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(54) Two-piece plastic holder for capped sample tubes

Zweiteilige Haltevorrichtung aus Kunststoff für mit Abdeckkappe versehene Probenröhrchen

Dispositif de retenue en matiere plastique en deux pièces pour des éprouvettes avec capuchon

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- **PATENT ABSTRACTS OF JAPAN vol. 6, no. 183 (P-143)18 September 1982 & JP-A-57 098 013 (OLYMPUS) 18 June 1982**

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Description

The invention pertains to the field of apparatus for performing the polymerase chain reaction (hereafter PCR). More particularly, the invention pertains to apparatus for use with automated instruments that can perform the polymerase chain reaction simultaneously on many samples with a very high degree of precision as to results obtained for each sample. This high precision provides the capability, among other things, of performing socalled "quantitative PCR".

To amplify DNA (Deoxyribose Nucleic Acid) using the PCR process, it is necessary to cycle a specially constituted liquid reaction mixture through a PCR protocol including several different temperature incubation periods. The reaction mixture is comprised of various components such as the DNA to be amplified and at least two primers selected in a predetermined way so as to be sufficiently complementary to the sample DNA as to be able to create extension products of the DNA to be amplified. The reaction mixture includes various enzymes and/or other reagents, as well as several deoxyribonucleoside triphosphates such as dATP, dCTP, dGTP and dTTP. Generally, the primers are oligonucleotides which are capable of acting as a point of initiation of synthesis when placed under conditions in which synthesis of a primer extension product which is complimentary to a nucleic acid strand is induced, i.e., in the presence of nucleotides and inducing agents such as thermostable DNA polymerase at a suitable temperature and pH.

The Polymerase Chain Reaction (PCR) has proven a phenomenally successful technology for genetic analysis, largely because it is so simple and requires relatively low cost instrumentation. A key to PCR is the concept of thermocycling: alternating steps of melting DNA, annealing short primers to the resulting single strands, and extending those primers to make new copies of double stranded DNA. In thermocycling, the PCR reaction mixture is repeatedly cycled from high temperatures (>90° C) for melting the DNA, to lower temperatures (40°C to 70°C) for primer annealing and extension. The first commercial system for performing the thermal cycling required in the polymerase chain reaction, the Perkin-Elmer Cetus DNA Thermal Cycler, was introduced in 1987.

Applications of PCR technology are now moving from basic research to applications in which large numbers of similar amplifications are routinely run. These areas include diagnostic research, biopharmaceutical development, genetic analysis, and environmental testing. Users in these areas would benefit from a high performance PCR system that would provide the user with high throughput, rapid turn-around time, and reproducible results. Users in these areas must be assured of reproducibility from sample-to-sample, run-to-run, lab-to-lab, and instrument-to-instrument.

For example, the physical mapping process in the Human Genome Project may become greatly simplified by utilizing sequence tagged sites (STS). An STS is a short, unique sequence easily amplified by PCR and which identifies a location on the chromosome. Checking for such sites to make genome maps requires amplifying large numbers of samples in a short time with protocols which can be reproductibly run throughout the world.

As the number of PCR samples increases, it becomes more important to integrate amplification with sample preparation and post-amplification analysis. The sample vessels must not only allow rapid thermal cycling but also permit more automated handling for operations such as solvent extractions and centrifugation. The vessels should work consistently at low volumes, to reduce reagent costs.

Generally PCR temperature cycling involves at least two incubations at different temperatures. One of these incubations is for primer hybridization and a catalyzed primer extension reaction. The other incubation is for denaturation, i.e., separation of the double stranded extension products into single strand templates for use in the next hybridization and extension incubation interval. The details of the polymerase chain reaction, the temperature cycling and reaction conditions necessary for PCR as well as the various reagents and enzymes necessary to perform the reaction are described in U.S. patents 4,683,202, 4,683,195, EPO Publication 258,017 and 4,889,818 (Taq polymerase enzyme patent) and all other PCR patents which are assigned to Cetus Corporation.

The purpose of a polymerase chain reaction is to manufacture a large volume of DNA which is identical to an initially supplied small volume of "seed" DNA. The reaction involves copying the strands of the DNA and then using the copies to generate other copies in subsequent cycles. Under ideal conditions, each cycle will double the amount of DNA present thereby resulting in a geometric progression in the volume of copies of the "target" or "seed" DNA strands present in the reaction mixture.

In some prior automated PCR instruments, the reaction mixture was stored in a disposable plastic tube which is closed with a cap. A typical sample volume for such tubes was approximately 100 microliters. Typically, such instruments used many such tubes filled with sample DNA and reaction mixture inserted into holes called sample wells in a metal block. To perform the PCR process, the temperature of the metal block was controlled according to prescribed temperatures and times specified by the user in a PCR protocol file. A computer and associated electronics then controlled the temperature of the metal block in accordance with the user supplied data in the PCR protocol file defining the times, temperatures and number of cycles, etc. As the metal block changed temperature, the samples in the various tubes followed with similar changes in temperature. However, in these prior art instruments not all samples experi-

enced exactly the same temperature cycle. In these prior art PCR instruments, errors in sample temperature were generated by nonuniformity of temperature from place to place within the metal sample block, i.e., temperature gradients existed within the metal of the block thereby causing some samples to have different temperatures than other samples at particular times in the cycle. Further, there were delays in transferring heat from the sample block to the sample, but the delays were not the same for all samples. To perform the PCR process successfully and efficiently, and to enable so called "quantitative" PCR, these time delays and temperature errors must be minimized to a great extent.

The problems of minimizing time delays for heat transfer to and from the sample liquid and minimizing temperature errors due to temperature gradients or nonuniformity in temperature at various points on the metal block become particularly acute when the size of the region containing samples becomes large. It is a highly desirable attribute for a PCR instrument to have a metal block which is large enough to accommodate 96 sample tubes arranged in the format of an industry standard microtiter plate.

The microtiter plate is a widely used means for handling, processing and analyzing large numbers of small samples in the biochemistry and biotechnology fields. Typically, a microtiter plate is a tray which is 9.2 cm (3 5/8 inches) wide and 12.7 cm (5 inches) long and contains 96 identical sample wells in an 8 well by 12 well rectangular array on 9 millimeter centers. Although microtiter plates are available in a wide variety of materials, shapes and volumes of the sample wells, which are optimized for many different uses, all microtiter plates have the came overall outside dimensions and the same 8 × 12 array of wells on 9 millimeter centers. A wide variety of equipment is available for automating the handling, processing and analyzing of samples in this standard microtiter plate format.

Generally microtiter plates are made of injection molded or vacuum formed plastic and are inexpensive and considered disposable. Disposability is a highly desirable characteristic because of the legal liability arising out of cross contamination and the difficulty of washing and drying microtiter plates after use.

It is therefore a highly desirable characteristic for a PCR instrument to be able to perform the PCR reaction on up to 96 samples simultaneously said samples being arranged in a microtiter plate format.

EP-A-0128778 (HSC Research Development Corporation) discloses a multiple well specimen dish primarily for use in cell culture studies. The dish includes a tray and a plurality of individual cells in the form of vessels which can be removably located in the tray. A lid is also provided for the tray and is physically identical therewith. Each vessel has a cover which is received in an opening in the lid when the tray and lid are assembled, so that pressure sensitive tape can then be used to releasably secure the covers to the lid while allowing one or more of the covers to be released when appropriate by peeling back the tape.

Accordingly, the present invention provides a two-piece plastic holder for loosely holding microtiter sample tubes of a preselected design, preferably up to 96 sample tubes, each having a cylindrically shaped upper section open at its top end and a closed, tapered lower section extending downwardly therefrom, each tube being of circular cross section and having a circumferential shoulder extending outwardly from said upper section at a position on said upper section spaced from the open end thereof, each tube having further a deformable cap for forming a gas-tight seal thereon, comprising:

a) a one-piece tray member comprising

i) a flat, horizontal plate section containing holes in an array compatible with industry standard microtiter plate format, preferably 96 holes in an 8-by-12 rectangular array, said holes being slightly larger than the outside diameter of the upper sections of said tubes but smaller than the outside diameter of said shoulder,

ii) a first vertical tray sidewall section completely around said plate extending upwardly to a height greater than the height of a tube resting in one of said holes,

iii) a second vertical tray sidewall section around said plate extending downwardly approximately to the bottom of the upper section of a tube resting in one of said holes,

b) a one-piece retainer releasably engageable inside said tray over any sample tubes resting in said tray comprising:

i) a flat, horizontal plate section containing holes in an array compatible with industry standard microtiter plate format, preferably 96 holes in an 8-by-12 rectangular array, said holes being slightly larger than the outside diameter of the upper sections of said tubes but smaller than the outside diameter of said shoulder,

ii) a vertical retainer sidewall section around said retainer plate section extending upwardly from said plate,

wherein when said retainer is engaged inside said tray, the retainer plate section lies slightly above the shoulder of a tube resting in said tray and the first tray sidewall section is about as high as said retainer sidewall section, whereby tubes resting in said tray are retained loosely both vertically and laterally and said caps project above said first vertical tray sidewall section but are downwardly deformable to the height of said section.

The design of the holder is such that each sample

tube advantageously has individual freedom of movement sufficient to find the best fit with the sample block under downward pressure from a heated cover, regardless of the number of sample tubes present. The microtiter plate design, by allowing each tube to find the best fit, provides high and uniform thermal conductance from the sample block to each sample tube even if differing rates of thermal expansion and contraction between the metal of the block and the plastic of the sample tube and microtiter plate structure cause the relative center-to-center dimensions of the wells in the sample block to change relative to the center-to-center distance of the sample tubes in the disposable microtiter plate structure.

The support mounting of the present invention holds sample tubes, preferably up to 96 sample tubes, in a microtiter plate format during the performance of PCR protocols using PCR apparatus having a corresponding sample block arrangement.

The plastic 96-well microtiter plate is preferably disposable. In use the tubes may contain DNA for thermal cycling.

The sample tube is preferably a thin walled sample tube for decreasing the delay between changes in sample temperature of the sample block and corresponding changes in temperature of the reaction mixture. Two different sample tube sizes can be used but each has a thin walled conical section that fits into a matching conical recess in the sample block. Typically, cones with 17° angles relative to the longitudinal axis may be used to prevent jamming of the tubes into the sample block but to allow snug fit. Other shapes and angles may suffice.

Heat exchangers other than sample blocks may be used e.g. liquid baths, ovens, etc. However, the wall thickness of the section of the sample tube which is in contact with whatever heat exchanger is being used is preferably as thin as possible so long as it is sufficiently strong to withstand the thermal stresses of PCR cycling and the stresses of normal use. Typically the sample tubes may be made of autoclavable polypropylene such as Himont PD701 with a wall thickness of the conical section in the range from 0.009 to 0.012 inches plus or minus 0.001 inches. Preferably, the wall thickness is 0.012 inches (1 inch = 2.54 cm).

The sample tube may also have a thicker walled cylindrical section which joins with the conical section. This conical section provide containment for the original reaction mixture or reagents which may be added after PCR processing.

The sample tube shown in Figure 31 has industry standard configuration except for the thin walls for compatibility in other PCR systems. The sample tube of Figure 2 is a shorter tube which may be used with the present invention.

The holes in the tray section are preferably countersunk and the underside of the shoulders of the tubes are correspondingly beveled.

The holes in the tray plate section and in the retainer plate section are preferably larger in diameter than the tubes by about 0.7mm.

The tray member preferably further comprises a plurality of support ribs extending along the underside of the tray plate member between rows of holes, the ribs extending downwardly to the same extent as the second vertical tray sidewall section.

The tray member preferably further comprises a skirt section extending at least partially around the tray plate section and depending vertically from that section, the skirt section being adapted to fit into a guard band groove in a thermocycler sample block.

Preferably the tray plate section has at least two openings provided therein and the retainer plate section has an identical number of vertical tabs, downwardly extending from the retainer plate, such that the tabs project through said openings and releasably engage the tray when the retainer is assembled with the tray.

The tabs may be disposed so as to form part of a skirt section extending downwardly at least partially around the tray plate section and wherein the tabs are adapted to fit into a guard band groove in a thermocycler sample block.

The openings and tabs may be positioned such that the retainer and the tray are capable of only one orientation relative to one another when the openings and the tabs are engaged.

The tabs are preferably deflectable in a sidewise direction in order to come into alignment with said openings.

The two-piece plastic holder may further comprise microtiter sample tubes preferably up to 96 tubes.

A preferred feature of the cap is that it has a downwardly depending cylindrical flange for forming a gas-tight seal with one of said tubes and a circumferential shoulder extending outwardly from the flange which prevents the flange from being seated on the tube below a predetermined point. The outer circumference of the downwardly depending flange will preferably fit snugly to form a gas-tight seal with the inner circumference of the tube.

Groups of 12 caps may be linked together to form a single strand of caps which are suitably spaced so as to form gas-tight seals with up to 12 tubes.

The caps are preferably deformable by heat and vertically downward force, more preferably resiliently deformable.

The gas-tight seal prevents loss of solvent from the reaction mixtures when the samples are being incubated at temperatures near their boiling point. In use, in performing PCR a heated platen may be used to cover the tops of the sample tubes. When the platen is in contact with an individual cap it provides a gas-tight seal for each sample tube; the heat from the platen heats the upper parts of each sample tube and the cap to a temperature above the condensation point such that no condensation and refluxing occurs within any sample tube. Condensation represents a relatively large heat transfer

since an amount of heat equal to the heat of vaporization is given up when water vapor condenses. This could cause large temperature variations from sample to sample if the condensation does not occur uniformly. The heated platen prevents any condensation from occurring in any sample tube thereby minimizing this source of potential temperature errors. The use of the heated platen also reduces reagent consumption.

Furthermore, the heated platen provides a downward force for each sample tube which exceeds an experimentally determined minimum downward force necessary to keep all sample tubes pressed firmly into the temperature controlled sample block so as to establish and maintain uniform block-to-tube thermal conductance for each tube. This uniformity of thermal conductance is established regardless of variations from tube to tube in length, diameter, angle or other dimensional errors which otherwise could cause some sample tubes to fit more snugly in their corresponding sample wells than other sample tubes.

The heated platen softens the plastic of each cap but does not totally destroy the caps elasticity. Thus, a minimum threshold downward force is successfully applied to each tube despite differences in tube height from tube to tube.

The two-piece plastic holder may further comprise a plastic base having wells arranged in an array, preferably 96 wells in an 8-by-12 rectangular array, the wells being dimensioned to snugly accept the lower sections of said sample tubes, preferably up to 96 tubes, the base being assemblable with the tray, the retainer and the tubes to form a microtiter plate having the footprint of an industry standard microtiter plate.

The invention will now be described by way of particular embodiments and with reference to the drawings in which:

Figure 1 is a cross-sectional view of a sample tube and cap seated in the sample block of the PCR apparatus, none of which form part of the invention.

Figure 2 is a cross-sectional view of a sliding cover and heated platen of a PCR apparatus, neither of which forms part of the invention.

Figure 3 is perspective view of the sliding cover, sample block of Figure 2 and a knob used to lower the heated platen, none of which form part of the invention.

Figure 4 is a cross-sectional view of the assembly of one embodiment of the frame, retainer, sample tube and cap when seated on a sample block.

Figure 5 is a cross-sectional view of the assembly of the preferred embodiment of the frame, retainer, sample tube and cap when seated on the sample block.

Figure 6 is a top, plan view of the plastic, disposable frame for the microtiter plate.

Figure 7 is a bottom, plan view of the frame.

Figure 8 is an end, elevation view of the frame.

Figure 9 is another end, elevation view of the frame.

Figure 10 is a cross-sectional view of the frame taken along section line 26-26' in Figure 6.

Figure 11 is a cross-sectional view of the frame taken along section line 27-27' in Figure 6.

Figure 12 is an edge elevation view and partial section of the frame.

Figure 13 is a sectional view of the preferred sample tube.

Figure 14 is a sectional view of the upper part of the sample tube.

Figure 15 is an elevation view of a portion of the cap strip.

Figure 16 is a top view of a portion of the cap strip.

Figure 17 is a top, plan view of the plastic, disposable retainer portion of the 96 well microtiter tray.

Figure 18 is a side, elevation view with a partial section of the retainer.

Figure 19 is an end, elevation view of the retainer.

Figure 20 is a sectional view of the retainer taken along section line 36-36' in Figure 17.

Figure 21 is a sectional view of the retainer taken along section line 37-37' in Figure 17.

Figure 22 is a plan view of the plastic disposable support base of the 96 well microtiter tray.

Figure 23 is a bottom plan view of the base.

Figure 24 is a side elevation view of the base.

Figure 25 is an end elevation view of the base.

Figure 26 is a sectional view of the support base taken along section line 42-42' in Figure 22.

Figure 27 is a sectional view of the support base taken along section line 43-43' in Figure 22.

Figure 28 is a section view of the base taken along section line 44-44' in Figure 22.

Figure 29 is a perspective exploded view of the plastic disposable items that comprise the microtiter tray with some sample tubes and caps in place.

Figure 30 is elevation sectional view of a tall thin walled sample tube marketed under the trademark MAXIAMP.

Figure 31 is a plan view of a sample tube and cap.

The figures which do not form part of the invention are presented because they assist in the description of the invention.

Sample mixtures including the DNA or RNA to be amplified are placed in the temperature-programmed sample block 12 of a computer directed instrument for performing PCR and are covered by heated cover.

The samples are stored in capped disposable tubes which are seated in the sample block and are thermally isolated from the ambient air by a heated cover which contacts a plastic disposable tray to be described below to form a heated, enclosed box in which the sample tubes reside. The heated cover serves, among other things, to reduce undesired heat transfers to and from the sample mixture by evaporation, condensation and refluxing inside the sample tubes. It also reduces the chance of cross contamination by keeping the insides of the caps dry thereby preventing aerosol formation when the tubes are uncapped. The heated cover is in contact with the sample tube caps and keeps them heat-

ed to a temperature of approximately 104°C or above the condensation points of the various components of the reaction mixture.

The purpose of the sample block is to provide a mechanical support and heat exchange element for an array of thin walled sample tubes where heat may be exchanged between the sample liquid in each sample tube and liquid coolant flowing in the bias cooling and ramp cooling channels formed in the sample block. Further, it is the function of the sample block to provide this heat exchange function without creating large temperature gradients between various ones of the sample wells such that all sample mixtures in the array experience the same PCR cycle even though they are spatially separated. The objective of the PCR instrument is to provide very tight temperature control over the temperature of the sample liquid for a plurality of samples such that the temperature of any sample liquid does not vary appreciably (approximately plus or minus 0.5°C) from the temperature of any other sample liquid in another well at any point in the PCR cycle.

There is an emerging branch of PCR technology called "quantitative" PCR. In this technology, the objective is to perform PCR amplification as precisely as possible by causing the amount of target DNA to exactly double on every cycle. Exact doubling on every cycle is difficult or impossible to achieve but tight temperature control helps.

There are many sources of errors which can cause a failure of a PCR cycle to exactly double the amount of target DNA (hereafter DNA should be understood as also referring to RNA) during a cycle. For example, in some PCR amplifications, the process starts with a single cell of target DNA. An error that can easily occur results when this single cell sticks to the wall of the sample tube and does not amplify in the first several cycles.

Another type of error is the entry of a foreign nuclease into the reaction mixture which attacks the "foreign" target DNA. All cells have some nonspecific nuclease that attacks foreign DNA that is loose in the cell. When this happens, it interferes with or stops the replication process. Thus, if a drop of saliva or a dandruff particle or material from another sample mixture were inadvertently to enter a sample mixture, the nuclease materials in these cells could attack the target DNA and cause an error in the amplification process. It is highly desirable to eliminate all such sources of cross-contamination.

Another source of error is nonprecise control over sample mixture temperature as between various ones of a multiplicity of different samples. For example, if all the samples are not precisely controlled to have the proper annealing temperature (a user selected temperature usually in the range from 50 to 60°C) for the extension incubation certain forms of DNA will not extend properly. This happens because the primers used in the extension process anneal to the wrong DNA if the temperature is too low. If the annealing termperature is too high, the primers will not anneal to the target DNA at all.

One can easily imagine the consequences of performing the PCR amplification process inaccurately when PCR amplification is part of diagnostic testing such as for the presence HIV antibodies, hepatitis, or the presence of genetic diseases such as sickle cell anemia, etc. A false positive or false negative result in such diagnostic testing can have disasterous personal and legal consequences. The design of any PCR should be such as to eliminate as much of these sources of possible errors as possible such as cross-contamination or poor temperature control while providing an instrument which is compatible with the industry standard 96-well microtiter plate format. The instrument must rapidly perform PCR in a flexible manner with a simple user interface.

The sample block is machined out of a solid block of relatively pure but corrosion resistant aluminium such as the 6061 aluminium alloy. Machining the block structure out of a solid block of aluminium results in a more thermally homogenous structure. Cast aluminium structures tend not to be as thermally homogenous as is necessary to meet the very tight desired temperature control specifications.

Sample block is capable of rapid changes in temperature because the thermal mass of the block is kept low. This is done by the formation in the block of many cooling passageways, sample wells, grooves and other threaded and unthreaded holes. Some of these holes are used to attach the block to supports and to attach external devices such as manifolds and spillage trays thereto.

The top surface of the sample block is drilled with an 8 x 12 array of conical sample wells. The walls of each sample well are drilled at an angle of 17° to match the angle of the conical section of each sample tube.

The bottom of each sample well includes a sump which has a depth which exceeds the depth of penetration of the tip of the sample tube. The sump is created by the pilot hole and provides a small open space beneath the sample tube when the sample tube is seated in the corresponding sample well. This sump provides a space for liquid such as condensation that forms on the well walls to reside without interfering with the tight fit of each sample tube to the walls of the sample well. This tight fit is necessary to insure that the thermal conductance from the well wall to the sample liquid is uniform and high for each sample tube. Any contamination in a well which causes a loose fit for one tube will destroy this uniformity of thermal conductance across the array. That is, because liquid is substantially uncompressible at the pressures involved in seating the sample tubes in the sample wells, if there were no sump , the presence of liquid in the bottom of the sample well could prevent a sample tube from fully seating in its sample well. Furthermore, the sump provides a space in which a gaseous phase of any liquid residing in the sump can expand during high temperature incubations such that large forces of such expansion which would be present if there

were no sump are not applied to the sample tube to push the tube out of flush contact with the sample well.

It has been found experimentally that it is important for each sample tube to be in flush contact with its corresponding sample well and that a certain minimum threshold force be applied to each sample tube to keep the thermal conductivity between the walls of the sample well and the reaction mixture uniform throughout the array. This minimum threshold seating force is shown as the force vector F in Figure 1 and is a key factor in preventing the thermal conductivity through the walls of one sample tube from being different than the thermal conductivity through the walls of another sample tube located elsewhere in the block. The minimum threshold seating force F is 30 grams and the preferred force level is between 50 and 100 grams.

The array of sample wells is substantially completely surrounded by a groove 78, best seen in Figures 4 and 5, which has two functions. The main function is to reduce the thermal conductivity from the central area of the sample block to the edge of the block. The groove 78 extends about 2/3 through the thickness of the sample block. This groove minimizes the effects of unavoidable thermal gradients caused by the necessary mechanical connections to the block of the support pins, manifolds, etc. A secondary function is to remove thermal mass from the sample block 12 so as to allow the temperature of the sample block 12 to be altered more rapidly and to simulate a row of wells in the edge region called the "guard band".

Isolation of the tubes from the ambient, and application of the minimum threshold force F pushing down on the sample tubes is achieved by a heated cover over the sample tubes and sample block.

Even though the sample liquid is in a sample tube pressed tightly into a temperature-controlled metal block, tightly capped, with a meniscus well below the surface of the temperature-controlled metal block, the samples still lose their heat upward by convection. Significantly, when the sample is very hot (the denaturation temperature is typically near the boiling point of the sample liquid), the sample liquid can lose a very significant amount of heat by refluxing of water vapor. In this process, water evaporates from the surface of the hot sample liquid and condenses on the inner walls of the cap and the cooler upper parts of the sample tube above the top surface of the sample block. If there is a relatively large volume of sample, condensation continues, and condensate builds up and runs back down the walls of the sample tube into the reaction mixture. This "refluxing" process carries about 2300 joules of heat per gram of water refluxed. This process can cause a drop of several degrees in the surface temperature of a 100 microliter reaction mixture thereby causing a large reduction of efficiency of the reaction.

If the reaction mixture is small, say 20 microliters, and the sample tube has a relatively large surface area above the top surface of the sample block, a significant fraction of the water in the reaction mixture may evaporate. This water may then condense inside the upper part of the sample tube and remain there by surface tension during the remainder of the high temperature part of the cycle. This can so concentrate the remaining reaction mixture that the reaction is impaired or fails completely.

In the prior art PCR thermal cyclers, this refluxing problem was dealt with by overlaying the reaction mixture with a layer of oil or melted wax. This immiscible layer of oil or wax floated on the aqueous reaction mixture and prevented rapid evaporation. However, labor was required to add the oil which raised processing costs. Further, the presence of oil interfered with later steps of processing and analysis and created a possibility of contamination of the sample. In fact, it is known that industrial grade mineral oils have in the past contaminated samples by the unknown presence of contaminating factors in the oil which were unknown to the users.

The need for an oil overlay is eliminated, and the problems of heat loss and concentration of the reaction mixture by evaporation and unpredictable thermal effects caused by refluxing are avoided according to the teachings of the invention by enclosing the volume above the sample block into which the upper parts of the sample tubes project and by heating this volume from above by a heated cover sometimes hereafter also called the platen.

Referring to Figure 2, there is shown a cross sectional view of the structure which is used to enclose the sample tubes and apply downward force thereto so as to supply the minimum threshold force F in Figure 1. A heated platen 14 is coupled to a lead screw 312 so as to move up and down along the axis symbolized by arrow 314 with rotation of the lead screw 312. The lead screw is threaded through an opening in a sliding cover 316 and is turned by a knob 318. The platen 314 is heated to a temperature above the boiling point of water by resistance heaters (not shown) controlled by a computer (not shown).

The sliding cover 316 slides back and forth along the Y axis on rails 320 and 322. The cover 316 includes vertical sides 317 and 319 and also includes vertical sides parallel to the X-Z plane (not shown) which enclose the sample block 12 and sample tubes. This structure substantially prevent drafts from acting on the sample tubes of which tubes 324 and 326 are typical.

Figure 3 is a perspective view of the sliding cover 316 and sample block 12 with the sliding cover in retracted position to allow access to the sample block. The sliding cover 316 resembles the lid of a rectangular box with vertical wall 328 having a portion 330 removed to allow the sliding cover 316 to slide over the sample block 12. The sliding cover is moved along the Y axis in Figure 3 until the cover is centered over the sample block 12. The user then turns the knob 318 in a direction to lower the heated platen 14 until a mark 332 on the knob 318

lines up with a mark 334 on an escutcheon plate 336. In some embodiments, the escutcheon plate 336 may be permanently affixed to the top surface of the sliding cover 316. In other embodiments, the escutcheon 336 may be rotatable such that the index mark 334 may be placed in different positions when different size sample tubes are used. In other words, if taller sample tubes are used, the heated platen 14 need not be lowered as much to apply the minimum threshold force F in Figure 1 . In use, the user screws the screw 318 to lower the platen 14 until the index marks line up. The user then knows that the minimum threshold force F will have been applied to each sample tube.

Referring jointly to Figures 1 and 2, prior to lowering the heated platen 14 in Figure 2, the plastic cap 338 for each sample tube sticks up about 0.5 millimeters above the level of the top of the walls of a plastic tray 340 (Figure 2) which holds all the sample tubes in a loose 8x12 array on 9 millimeter centres. The array of sample wells can hold up to 96 MicroAmp™ PCR tubes of 100 µl capacity or 48 larger GeneAmp™ tubes of 0.5 ml capacity. The details of this tray will be discussed in greater detail below.

The tray 340 has a planar surface having an 8x12 array of holes for sample tubes. This planar surface is shown in Figures 1 and 2 as a horizontal line which intersects the sample tubes 324 and 326 in Figure 2. Tray 340 also has four vertical walls two of which are shown at 342 and 344 in Figure 2. The top level of these vertical walls, shown at 346 in Figure 1, establishes a rectangular box which defines a reference plane.

As best seen in Figure 1, the caps 338 for all the sample tubes project above this reference plane 346 by some small amount which is designed to allow the caps 338 to be softened and deformed by the heated platen 14 and "squashed" down to the level of the reference plane 346. The heated platen 14 is kept at a temperature of 105°C. The knob 318 in Figure 2 and the lead screw 312 are turned until the heated platen 14 descends to and makes contact with the tops of the caps 338. The caps 338 for the sample tubes are made of polypropylene. These caps soften shortly after they come into contact with the heated platen 14. As the caps soften, they deform, but they do not lose all of their elasticity. After contacting the caps, the heated platen is lowered further until it rests upon the reference plane 346. This further lowering deforms the caps 338 and causes a minimum threshold force F of at least 50 grams to push down on each sample tube to keep each tube well seated firmly in its sample well. The amount by which the caps 338 project above the reference plane 346, and the amount of deformation and residual elasticity when the heated platen 14 rests upon the reference plane 346 is designed such that a minimum threshold force F of at least 50 grams and preferably 100 grams will have been achieved for all sample tubes then present after the heated platen 14 has descended to the level of the reference plane 346.

The heated platen 14 and the four vertical walls and planar surface of the tray 340 form a heated, sealed compartment when the platen 14 is in contact with the top edge 346 of the tray. The plastic of the tray 340 has a relatively poor thermal conductivity property. It has been found experimentally that contacting the heated platen 14 with the caps 338 and the isolation of the portion of the sample tubes 288 which project above the top level 280 of the sample block 12 by a wall of material which has relatively poor thermal conductivity has a beneficial result. With this structure, the entire upper part of the tube and cap are brought to a temperature which is high enough that little or no condensation forms on the inside surfaces of the tube and cap since the heated platen is kept at a temperature above the boiling point of water. This is true even when the sample liquid 276 in Figure 1 is heated to a temperature near its boiling point. This eliminates the need for a layer of immiscible material such as oil or wax floating on top of the sample mixture 276 thereby reducing the amount of labour involved in a PCR reaction and eliminating one source of possible contamination of the sample.

It has been found experimentally that in spite of the very high temperature of the heated cover and its close proximity to the sample block 12, there is little affect on the ability of the sample block 12 to cycle accurately and rapidly between high and low temperatures.

The heated platen 14 prevents cooling of the samples by the refluxing process noted earlier because it keeps the temperature of the caps above the condensation point of water thereby keeping the insides of the caps dry. This also prevents the formation of aerosols when the caps are removed from the tubes.

The sample tubes may vary by a few thousandths of an inch in their overall height. Further, the caps for the sample tubes may also vary in height by a few thousandths of an inch. Also, each conical sample well in the sample block 12 may not be drilled to exactly the same depth, and each conical sample well in the sample block may be drilled to a slightly different diameter and angle. Thus, when a population of capped tubes is placed in the sample block so as to be seated in the corresponding sample well, the tops of the caps will not all necessarily be at the same height. The worst case discrepancy for this height could be as much as 0.5 millimeters from the highest to the lowest tubes.

If a perfectly flat unheated platen 14 mounted so that it is free to find its own position were to be pressed down on such an array of caps, it would first touch the three tallest tubes. As further pressure was applied and the tallest tubes were compressed somewhat, the platen would begin to touch some caps of lower tubes. There is a distinct possibility that unless the tube and cap assemblies were compliant, the tallest tubes would be damaged before the shortest tubes were contacted at all. Alternatively, the force necessary to compress all the tall tubes sufficiently so as to contact the shortest tube could be too large for the device to apply. In either

case, one or more short tubes might not be pressed down at all or might be pressed down with an insufficient amount of force to guarantee that the thermal time constant for that tube was equal to the thermal time constants for all the other tubes. This would result in the failure to achieve the same PCR cycle for all tubes in the sample block since some tubes with different thermal time constants would not be in step with the other tubes. Heating the platen and softening the caps eliminates these risks by eliminating the manufacturing tolerance errors which lead to differing tube heights as a factor.

The entire heated platen can be covered with a compliant rubber layer. A compliant rubber layer on the heated platen would solve the height tolerance problem, but would also act as a thermal insulation layer which would delay the flow of heat from the heated platen to the tube caps. Further, with long use at high temperatures, most rubber materials deteriorate or become hard. It is therefore desirable that the heated platen surface be a metal and a good conductor of heat.

Alternatively, 96 individual springs could be mounted on the platen so that each spring individually presses down on a single sample tube. This is a complex and costly solution, however, and it requires that the platen be aligned over the tube array with a mechanical precision which would be difficult or bothersome to achieve.

The necessary individual compliance for each sample tube in the preferred embodiment is supplied by the use of plastic caps which collapse in a predictable way under the force from the platen but which, even when collapsed, still exert a downward force F on the sample tubes which is adequate to keep each sample tube seated firmly in its well.

In the sample tube cap 338 shown in Figure 1, the surface 350 should be free of nicks, flash and cuts so that it can provide a hermetic seal with the inner walls 352 of the sample tube 288. The preferred material for the cap is polypropylene. A suitable material might be Valtec HH-444 or PD701 polypropylene manufactured by Himont as described above or PPW 1780 by American Hoescht. The preferred wall thickness for the domed portion of the cap is 3.302 + .000 - 0.127 mm (0.130 + .000 - 0.005 inches). The thickness of the shoulder portion 356 is 0.635 mm (0.025 inches) and the width of the domed shaped portion of the cap is 5.156 mm (0.203 inches) in the preferred embodiment.

Any material and configuration for the caps which will cause the minimum threshold force F in Figure 1 to be applied to all the sample tubes and which will allow the cap and upper portions of the sample tubes to be heated to a temperature high enough to prevent condensation and refluxing will suffice. The dome shaped cap 338 has a thin wall to aid in deformation of the cap. Because the heated platen is kept at a high temperature, the wall thickness of the domed shape cap can be thick enough to be easily manufactured by injection moulding since the necessary compliance to account for differences in tube height is not necessary at room temperature.

The platen can be kept at a temperature anywhere from 94°C to 110° although the range from 100°C to 110°C is preferred to prevent refluxing since the boiling point of water is 100°C.

In this temperature range, it has been experimentally found that the caps soften just enough to collapse easily by as much as 1 millimeter. Studies have shown that the elastic properties of the polypropylene used are such that even at these temperatures, the collapse is not entirely inelastic. That is, even though the heated platen causes permanent deformation of the caps, the material of the caps still retain a significant enough fraction of their room temperature elastic modulus that the minimum threshold force F is applied to each sample tube. Further, the heated platen levels all the caps that it contacts without excessive force regardless of how many tubes are present in the sample block because of the softening of the cap.

Because the cap temperature is above the boiling point of water during the entire PCR cycle, the inside surfaces of each cap remain completely dry. Thus, at the end of a PCR process, if the samples are cooled to room temperature before being removed from the sample block, if the caps on each sample tube are opened, there is no possibility of creating an aerosol spray of the sample tube contents which could result in cross contamination. This is because there is no liquid at the cap to tube seal when the seal is broken.

This is extremely advantageous, because tiny particles of aerosol containing amplified product DNA can contaminate a laboratory and get into sample tubes containing samples from other sources, e.g., other patients, thereby possibly causing false positive or negative diagnostic results which can be very troublesome. Users of the PCR amplification process are extremely concerned that no aerosols that can contaminate other samples be created.

A system of disposable plastic items is used to convert the individual sample tubes to an 8x12 array which is compatible with microtiter plate format lab equipment but which maintains sufficient individual freedom of movement to compensate for differences in the various rates of thermal expansion of the system components. The relationship of the thermally compliant cap to the rest of this system is best seen in Figure 4 which is a cross sectional view of the sample block, and two sample tubes with caps in place with the sample tubes being held in place by the combination of one embodiment of a plastic 96 well microtiter tray and a retainer. Figure 5 is an alternative, preferred embodiment showing the structure and interaction of most of the various plastic disposable items of the system. The rectangular plastic 96 well microtiter plate tray 342 rests on the surface of the sample block 12. The top edge 346 of the frame 342 has a height which is approximately 0.5 millimeters shorter than the height of the caps of which cap 364 is

exemplary. All of the capped tubes will project higher than the edge 346 of the frame 342. The frame 342 is configured such that a downward extending ridge 366 extends into the guardband groove 78 through its entire length. The frame 342 does however have a gap (not shown) which corresponds to the gap in the groove 78 for a temperature sensor (not shown).

The reference plane 346 mentioned above is established by the top of the frame 342. How this reference plane interacts with the heated platen is as follows. Prior to screwing down the knob 318 in Figure 3 to line up the index marks 332 and 334 to start an amplification run, a calibration process will have been performed to locate the position of the index mark on the escutcheon platen 336 in Figure 3.. This calibration is started by placing the frame 342 in Figure 4 in position on the sample block. The frame 342 will be empty however or any sample tubes therein will not have any caps in place. Then, the knob 318 is screwed down until the heated platen 14 is firmly in contact with the top edge 346 of the frame 342 around its entire parameter. When the knob 318 has been screwed down sufficiently to allow the heated platen to rest on the reference plane 346 and to press the frame 342 firmly against the top surface 280 of the sample block, the rotatable escutcheon 336 of the preferred embodiment will be rotated until the index mark 334 on the escutcheon lines up with the index mark 332 on the knob 318. Then, the knob 318 is rotated counterclockwise to raise the platen 14 and the cover 316 in Figure 2 is slid in the negative Y direction to uncover the frame 342 and the sample block 12. Sample tubes with caps loaded with a sample mixture may then be placed in position in the frame 342. The heated cover 316 is then placed back over the sample block, and the knob 318 is turned clockwise to lower the heated platen 14 until the index mark 332 on the knob lines up with the index mark 334 as previously positioned. This guarantees that all tubes have been firmly seated with the minimum force F applied. The use of the index marks gives the user a simple, verifiable task to perform.

If there are only a few sample tubes in place, it will take only a small amount of torque to line up the index marks 332 and 334. If there are many tubes, however, it will take more torque on the knob 318 to line up the index marks. This is because each tube is resisting the downward movement of the heated platen 14 as the caps deform. However, the user is assured that when the index marks 332 and 334 are aligned, the heated platen will once again be tightly placed against the top edge 346 of the frame 342 and all tubes will have the minimum threshold force F applied thereto. This virtually guarantees that the thermal time constant for all the tubes will be substantially the same.

In alternative embodiments, the index marks 332 and 334 may be dispensed with, and the knob 318 may simply be turned clockwise until it will not turn any more. This condition will occur when the heated platen 14 has reached the top edge or reference plane 346 and the plastic frame 342 has stopped further downward movement of the heated platen 14. Obviously in this alternative embodiment, and preferably in the index mark embodiment described above, the plastic of the frame 342 will have a melting temperature which is sufficiently high to prevent deformation of the plastic of the frame 342 when it is in contact with the heated platen 14. In the preferred embodiment, the plastic of the frame 342 is celanese nylon 1503 with a wall thickness of 1.27 mm (0.05 inches).

An advantage of the above described system is that sample tubes of different heights may be used simply by using frames 342 having different heights. The frame 342 should have a height which is approximately 0.5 millimeters shorter than the plane of the tips of the capped tubes when both are seated in the sample block. In the preferred embodiment, two different tube heights are used. The range of motion of the lead screw 312 which drives the heated platen 14 in Figure 2 must be sufficient for all the different sizes of sample tubes to be used. Of course, during any particular PCR processing cycle, all tubes must be the same height.

The system described above provides uniform temperatures in the sample block, uniform thermal conductance from block to sample, and isolation of the sample tubes from the vagaries of the ambient environment. Any number of sample tubes up to 96 may be arrayed in the microtiter plate format. The system allows accurate temperature control for a very large number of samples and a visual indication of the sample temperatures for all samples without actually measuring the temperature of any sample.

As the container for PCR reactions, it has been common in the prior art to use polypropylene tubes which were originally designed for microcentrifuges. This prior art tube had a cylindrical cross-section closed at the top by a snap-on cap which makes a gas-tight seal. This prior art tube had a bottom section which comprised the frustrum of a cone with an included angle of approximately 17 degrees.

When such a conical sample tube is pressed down into a sample well of a sample block with a conical cavity with the same included angle, and when the sample mixture in the tube lies entirely within the conical volume and below the top surface of the sample block, the thermal conductance between the block and the liquid can be made adequately predictable for good uniformity of sample temperature throughout the array. To achieve adequate control of the thermal conductance between the sample block and the sample mixture, the included angles of the conical tube and the sample well must match closely, and the conical surfaces of the tube and well must be smooth and held together in flush relation. Further, the minimum threshold force F must be applied to each sample tube to press each tube tightly into the sample well so that it does not rise up or loosen in the well for any reason during thermal cycling, such as steam formation from trapped liquid in space 291 in Fig-

ure 1 . Finally, each tube must be loaded with the same amount of sample liquid. If the above listed conditions are met, the thermal conductance between the sample block and the sample liquid in each tube will be predominantly determined by the conductance of the conical plastic wall 368 in Figure 15 and a boundary layer, (not shown) of the sample liquid at the inside surface 370 of the conical sample tube wall.

The thermal conductance of the plastic tube walls is determined by their thickness, which can be closely controlled by the injection moulding method of manufacture of the tubes. The sample liquid in all the sample tubes has virtually identical thermal properties.

It has been found by experiment and by calculation that a moulded, one-piece, 96-well microtiter plate is only marginally feasible for PCR because the differences in the thermal expansion coefficients between aluminium and plastic lead to dimensional changes which can destroy the uniformity of thermal conductance to the sample liquid across the array. That is, since each well in such a one-piece plate is connected to each other well through the surface of the plate, the distances between the wells are determined at the time of initial manufacture of the plate but change with changing temperature since the plastic of the plate has a significant coefficient of thermal expansion. Also, distances between the sample wells in the metal sample block 12 are dependent upon the temperature of the sample block since aluminium also has a significant coefficient of thermal expansion which is different than that of plastic. To have good thermal conductance, each sample well in a one-piece 96-well microtiter plate would have to fit almost perfectly in the corresponding well in the sample block at all temperatures. Since the temperature of the sample block changes over a very wide range of temperatures, the distances between the sample wells in the sample block vary cyclically during the PCR cycle. Because the coefficients of thermal expansion for plastic and aluminium are substantially different, the distances of the well separation in the sample block would vary differently over changing temperatures than would the distances between the sample wells of a plastic, one-piece, 96-well microtiter plate.

Thus, as an important criteria for a perfect fit between a sample tube and the corresponding sample well over the PCR temperature range, it is necessary that each sample tube in the 96-well array be individually free to move laterally and each tube must be individually free to be pressed down vertically by whatever amount is necessary to make flush contact with the walls of the sample well.

The sample tubes preferred for use in the invention are different from the known microcentrifuge tubes in that the wall thickness of the conical frustrum position of the sample tube is much thinner to allow faster heat transfer to and from the sample liquid. The upper part of these tubes has a thicker wall thickness than the conical part. In Figure 1, the wall thickness in the cylindrical part 288 in Figure 1 is generally 0.762 mm (0.030 inches) while the wall thickness for the conical wall 368 is 0.229 mm (0.009 inches). Because thin parts cool faster than thick parts in the injection moulding process, it is important to get the mould full before the thin parts cool off.

Conventional injection moulding techniques and mould manufacture techniques for the injection mould will suffice for purposes of providing suitable tubes.

The use of cone shaped sample tubes translates substantially all manufacturing tolerance errors to height errors, i.e., a variance from tube to tube in the height of the tip of the cap to the top of the sample block when the sample tube is seated in the sample well. For example, an angle error for the angle of the sample tube walls is converted to a height error when the tube is placed in the sample block because of the mismatch between the tube wall angle and the sample well wall angle. Likewise, a diameter error in the dimensions of the cone would also translate into a height error since the conical part of the tube would either penetrate deeper or not as much as a properly dimensional tube.

For good uniformity of thermal conductance across the array, a good fit between the sample tubes and the sample well must exist for all 96-wells over the full temperature range of 0 to 100°C regardless of differences in thermal expansion rates. Also, each of the 96 sample tubes must have walls with dimensions and wall thicknesses which are uniform to a very high degree. Each sample tube in which sample mixture is to be held should be fitted with a removable gas-tight cap that makes a gas-tight seal to prevent loss of water vapor from the reaction mixture when this mixture is at or near its boiling point such that the volume of the sample mixture does not decrease. All these factors combine to make a one-piece microtiter plate with 96 individual sample wells extremely difficult to manufacture in a manner so as to achieve uniform thermal conductance for all 96 wells.

Any structure which provides the necessary individual lateral and vertical degrees of freedom for each sample tube will suffice for purposes of practicing the invention.

According to the teachings of the preferred embodiment of the invention, all the above noted requirements have been met by using a 4 piece disposable plastic system. This system gives each sample tube sufficient freedom of motion in all necessary directions to compensate for differing rates of thermal expansion and yet retains up to 96 sample tubes in a 96 well microtiter plate format for user convenience and compatibility with other laboratory equipment which is sized to work with the industry standard 96-well microtiter plate. The multi-piece disposable plastic system is very tolerant of manufacturing tolerance errors and the differing thermal expansion rates over the wide temperature range encountered during PCR thermal cycling.

Figures 4 and 5 show alternative embodiments of

most of the four piece plastic system components in cross-section as assembled to hold a plurality of sample tubes in their sample wells with sufficient freedom of motion to account for differing rates of thermal expansion. Figure 28 shows all the parts of the disposable plastic microtiter plate emulation system in an exploded view. This figure illustrates how the parts fit together to form a microtiter plate with all the sample tubes loosely retained in an 8x12 microtiter plate format 96 well array. Figure 6 shows a plan view of a microtiter plate frame 342 according to the teachings of the invention which is partially shown in cross-section in Figures 4 and 5 . Figure 7 shows a bottom view plan view of the frame 342. Figure 8 is an end view of the frame 342 taken from view line 24-24' in Figure 6 . Figure 9 is an end view of the frame 342 taken from view line 25-25' in Figure 6 . Figure 10 is a cross section through the frame 342 at section line 26-26' in Figure 6. Figure 11 is a cross sectional view through the frame 342 taken along section line 27-27' in Figure 6. Figure 12 is a side view of the frame 342 taken along view line 28-28' in Figure 6 with a partial cut away to show in more detail the location where a retainer to be described below clips to the frame 342.

Referring jointly to Figures 4 , 5 and 6 through 12, the frame 342 is comprised of a horizontal plastic plate 372 in which there are formed 96 holes spaced on 9 millimeter centres in the standard microtiter plate format. There are 8 rows labeled A through H and 12 columns labeled 1 through 12. Hole 374 at row D, column 7 is typical of these holes. In each hole in the frame 342 there is placed a conical sample tube such as the sample tube 376 shown in Figure 1. Each sample tube is smaller in diameter than the hole in which it is placed by about 0.7 millimeters, so that there is a loose fit in the hole. This is best seen in Figures 4 and 5 by observing the distance between the inside edge 378 of a typical hole and the side wall 380 of the sample tube placed therein. Reference numeral 382 in Figures 4 and 5 shows the opposite edge of the hole which is also spaced away from the outside wall of the cylindrical portion of the sample tube 376.

Each sample tube has a shoulder shown at 384 in Figures 1 , 4 and 5. This shoulder is moulded around the entire circumference of the cylindrical portion 288 of each sample tube. The diameter of this shoulder 384 is large enough that it will not pass through the holes in the frame 342, yet not so large as to touch the shoulders of the adjacent tubes in neighboring holes.

Once all the tubes are placed in their holes in the frame 342, a plastic retainer 386 (best seen in Figures 4 and 5 and Figure 28) is snapped into apertures in the frame 342. The purpose of this retainer is to keep all the tubes in place such that they cannot fall out or be knocked out of the frame 342 while not interfering with their looseness of fit in the frame 342. The retainer 386 is sized and fitted to the frame 342 such that each sample tube has freedom to move vertically up and down to some extent before the shoulder 384 of the tube encounters either the retainer 386 or the frame 342. Thus, the frame and retainer, when coupled, provide a microtiter plate format for up to 96 sample tubes but provide sufficient horizontal and vertical freedom such that each tube is free to find its best fit at all temperatures under the influence of the minimum threshold force F in Figure 1.

A more clear view of the sample tube and shoulder may be had by reference to Figures 13 and 14. Figures 13 and 14 are an elevation sectional view and a partial upper section of the shoulder portion, respectively, of a typical sample tube. A plastic dome-shaped cap such as will be described in more detail below is inserted into the sample tube shown in Figure 13 and forms a hermetic seal with the inside wall 390 of the top at the sample tube. A ridge 392 formed in the inside wall of the sample tube acts as a stop for the dome-shaped cap to prevent further penetration. Normally, the dome-shaped caps come in strips connected by web.

Figure 15 shows three caps in elevation view connected by a web 394 and terminated in a tab 396. The tab aids the user in removing an entire row of caps by a single pull. Normally, the web 394 rests on the top surface 398 of the sample tube and prevents further penetration of the cap into the sample tube. Each cap includes a ridge 400 which forms the hermetic seal between the cap and the inside wall of the sample tube. Figure 16 shows a top view of three caps in a typical strip of 12 connected caps.

For a more detailed understanding of the retainer, refer to Figures 17 through 21. Figure 17 is a top view of the plastic retainer. Figure 18 is an elevation view of the retainer taken along view line 34-34' in Figure 17. Figure 19 is an end elevation view of the retainer taken along view line 35-35' in Figure 17. Figure 20 is a sectional view taken along section line 36-36' in Figure 17. Figure 21 is a sectional view through the retainer taken along section line 37-37' in Figure 17.

Referring jointly to Figures 17-21, the retainer 386 is comprised of a single horizontal plastic plane 402 surrounded by a vertical wall 404. The plane 402 has an 8 x 12 array of 96 holes formed therein divided into 24 groups of four holes per group. These groups are set off by ridges formed in the plane 402 such as ridges 406 and 408. Each hole, of which hole 410 is typical, has a diameter D which is larger than the diameter D_1 in Fig. 13 and smaller than the diameter D_2. This allows the retainer to be slipped over the sample tubes after they have been placed in the frame 342 but prevents the sample tubes from falling out of the frame since the shoulder 384 is too large to pass through the hole 410.

The retainer snaps into the frame 342 by means of plastic tabs 414 shown in Figures 18 and 20. These plastic tabs are pushed through the slots 416 and 418 in the frame as shown in Figure 7. There are two plastic tabs 414, one on each long edge of the retainer. These two plastic tabs are shown as 414A and 414B in Figure 17.

The frame 342 of Figures 6-12, with up to 96 sample tubes placed therein and with the retainer 386 snapped into place, forms a single unit such as is shown in Figures 4 and 5 which can be placed in the sample block 12 for PCR processing.

After processing, all the tubes may be removed simultaneously by lifting the frame 342 out of the sample block. For convenience and storage, the frame 342 with sample tubes and retainer in place can be inserted into another plastic component called the base. The base has the outside dimensions and footprint of a standard 96-well microtiter plate and is shown in Figures 22 through 29. Figure 22 is a top plan view of the base 420, while Figure 23 is a bottom plan view of the base. Figure 24 is an elevation view of the base taken from view line 40-40' in Figure 22. Figure 25 is an end elevation view taken from view line 41-41' in Figure 22. Figure 26 is a sectional view taken through the base along section line 42-42' in Figure 22. Figure 27 is a sectional view through the base taken along section line 43-43' in Figure 22. Figure 29 is a sectional view taken along section line 44-44' in Figure 22.

The base 420 includes a flat plane 422 of plastic in which an 8 x 12 array of holes with sloped edges is formed. These holes have dimensions and spacing such that when the frame 342 is seated in the base, the bottoms of the sample tubes fit into the conical holes in the base such that the sample tubes are held in the same relationship to the frame 342 as the sample tubes are held when the frame 342 is mounted on the sample block. Hole 424 is typical of the 96 holes formed in the base and is shown in Figures 38, 44 and 43. The individual sample tubes, though loosely captured between the tray and retainer, become firmly seated and immobile when the frame is inserted in the base. The manner in which a typical sample tube 424 fits in the base is shown in Figure 29.

In other words, when the frame, sample tubes and retainer are seated in the base 420 the entire assembly becomes the exact functional equivalent of an industry standard 96-well microtiter plate, and can be placed in virtually any automated pipetting or sampling system for 96- well industry standard microtiter plates for further processing.

After the sample tubes have been filled with the necessary reagents and DNA sample to be amplified, the sample tubes can be capped. In an alternative embodiment of the cap strip shown in Figures 15 and 16, an entire mat of 96 caps with a compliant web connecting them in an 8 x 12 array may be used. This web, shown at 394 in Figure 15 must be sufficiently compliant so that the caps do not restrain the sample tubes from making the small motions these sample tubes must make to fit perfectly in the conical wells of the sample block at all temperatures.

The assembly of tubes, caps frames, retainer and base is brought after filling the tubes to the thermal cycler. There, the frame, capped tubes and retainer plate are removed from the base as a unit. This unit is then placed in the sample block 12 to make the assembly shown in Figure 4 or 5 with the tubes loosely held in the conical wells in the sample block. As shown in Figure 4 and 5, the frame 342 is seated on the top surface 280 of the guardband. In the preferred embodiment, the ridge 366 extends down into the groove 78 of the guardband, but this is not essential.

Next, the heated cover is slid over the samples, and the heated platen is screwed down as previously described until it contacts the top edge 346 of the frame 342.

Within seconds after the heated platen 14 in Figure 2 touches the caps, the caps begin to soften and yield under the downward pressure from the lead screw 312 in Figure 2. The user then continues to turn to knob 318 until the index marks 332 and 334 in Figure 3 line up which indicates that every sample tube has been tightly pressed into the sample block with at least the minimum threshold force F and all air gaps between the heated platen 14, the sample block and the top edge 346 of the frame 342 have been tightly closed. The sample tubes are now in a completely closed and controlled environment, and precision cycling of temperature can begin.

At the end of the PCR protocol, the heated platen 14 is moved upward and away from the sample tubes, and the heated cover 316 is slid out of the way to expose the frame 342 and sample tubes. The frame, sample tubes and retainer are then removed and replaced into an empty base, and the caps can be removed. As each cap or string of caps is pulled off, the retainer keeps the tube from coming out of the tray. Ribs formed in the base (not shown in Figures 22-29) contact the retainer tabs 414A and 414B shown in Figure 17 to keep the retainer snapped in place such that the force exerted on the tubes by removing the caps does not dislodge the retainer 386.

Obviously, the frame 342 may be used with fewer than 96 tubes if desired. Also, the retainer 386 can be removed if desired by unsnapping it.

A user who wishes to run only a few tubes at a time and handle these tubes individually can place an empty frame 342 without retainer on the sample block. The user may then use the base as a "test tube rack" and set up a small number of tubes therein. These tubes can then be filled manually and capped with individual caps. The user may then transfer the tubes individually into wells in the sample block, close the heated cover and screw down the heated platen 14 until the marks line up. PCR cycling may then commence. When the cycling is complete, the cover 316 is removed and the sample tubes are individually placed in an available base. The retainer is not necessary in this type of usage.

Claims

1. A two-piece plastic holder (342,386) for loosely

holding microtiter sample tubes (376) of a preselected design, preferably up to 96 sample tubes, each having a cylindrically shaped upper section (288) open at its top end and a closed, tapered lower section extending downwardly therefrom, each tube being of circular cross section and having a circumferential shoulder (384) extending outwardly from said upper section at a position on said upper section spaced from the open end thereof, each tube having further a deformable cap (364) for forming a gas-tight seal thereon, comprising:

a) a one-piece tray member (342) comprising

i) a flat, horizontal plate section (372) containing holes (374) in an array compatible with industry standard microtiter plate format, preferably 96 holes in an 8-by-12 rectangular array, said holes being slightly larger than the outside diameter of the upper sections of said tubes but smaller than the outside diameter of said shoulder,
ii) a first vertical tray sidewall section (346) completely around said plate extending upwardly to a height greater than the height of a tube resting in one of said holes,
iii) a second vertical tray sidewall section (346) around said plate extending downwardly approximately to the bottom of the upper section of a tube resting in one of said holes,

b) a one-piece retainer (386) releasably engageable inside said tray (342) over any sample tubes resting in said tray comprising:

i) a flat, horizontal plate section (402) containing holes (410) in an array compatible with industry standard microtiter plate format, preferably 96 holes in an 8-by-12 rectangular array said holes being slightly larger than the outside diameter of the upper sections of said tubes but smaller than the outside diameter of said shoulder,
ii) a vertical retainer sidewall section around said retainer plate section extending upwardly from said plate,

wherein when said retainer (386) is engaged inside said tray (342), the retainer plate section lies slightly above the shoulder of a tube resting in said tray and the first tray sidewall section is about as high as said retainer sidewall section, whereby tubes resting in said tray are retained loosely both vertically and laterally and said caps project above said first vertical tray sidewall section but are downwardly deformable to the height of said section.

2. A holder as claimed in claim 1 wherein the holes in said tray section are countersunk and wherein the underside of the shoulders of said tubes are correspondingly beveled.

3. A holder as claimed in claim 2 wherein the holes in the tray plate section and in the retainer plate section are larger in diameter than said tubes by about 0.7mm.

4. A holder as claimed in any one of claims 1 to 3 wherein said tray member further comprises a plurality of support ribs extending along the underside of the tray plate member between rows of holes said ribs (406, 408) extending downwardly to the same extent as said second vertical tray sidewall section.

5. A holder as claimed in any preceding claim wherein said tray member further comprises a skirt section (366) extending at least partially around said tray plate section and depending vertically from that section, said skirt section being adapted to fit into a guard band (78) groove in a thermocycler sample block (12).

6. A holder as claimed in any preceding claim wherein said tray plate section has at least two openings (416, 418) provided therein and said retainer plate section has an identical number of vertical tabs (414A, 414B), downwardly extending from said retainer plate, such that said tabs project through said openings and releasably engage the tray when said retainer is assembled with said tray.

7. A holder as claimed in claim 6 wherein said tabs are disposed so as to form part of a skirt section extending downwardly at least partially around said tray plate section and wherein said tabs are adapted to fit into a guard band groove in a thermocycler sample block.

8. A holder as claimed in claim 7 wherein said openings and said tabs are positioned such that said retainer and said tray are capable of only one orientation relative to one another when said openings and said tabs are engaged.

9. A holder as claimed in any one of claims 6 to 8 wherein said tabs are deflectable in a sidewise direction in order to come into alignment with said openings.

10. A holder as claimed in any preceding claim further comprising microtiter sample tubes in said holder, preferably up to 96 tubes.

11. A holder as claimed in any preceding claim wherein each said cap has a downwardly depending cylin-

drical flange for forming a gas-tight seal with one of said tubes and a circumferential shoulder extending outwardly from said flange which prevents said flange from being seated on the tube below a predetermined point.

12. A holder as claimed in claim 11 wherein the outer circumference of said downwardly depending flange fits snugly to form a gas-tight seal with the inner circumference of said tube.

13. A holder as claimed in any preceding claim wherein groups of 12 of said caps are linked together to form a single strand of caps which are suitably spaced so as to form gas-tight seals with up to 12 of said tubes.

14. A holder as claimed in any preceding claim wherein said caps are deformable by heat and vertically downward force.

15. A holder as claimed in any preceding claim wherein said caps are resiliently deformable.

16. A holder as claimed in any preceding claim further comprising a plastic base (420) having wells arranged in an array, preferably 96 wells in a rectangular array, said wells being dimensioned to snugly accept the lower sections of said sample tubes, preferably up to 96 tubes, said base being assemblable with said tray, said retainer and said tubes to form a microtiter plate having the footprint of an industry standard microtiter plate.

Patentansprüche

1. Zweiteiliger Plastik-Halter (342, 386) zum losen Halten von Mikrotiter-Proben-Röhrchen (376) eines vorgewählten Designs, vorzugsweise bis zu 96 Proben-Röhrchen, wobei ein jedes Proben-Röhrchen einen zylindrisch geformten unteren Abschnitt (288), der an seinem oberen Ende offen ist, aufweist sowie einen geschlossenen, konisch zulaufenden unteren Abschnitt, der sich davon nach unten hin erstreckt, wobei jedes Röhrchen einen kreisförmigen Querschnitt sowie einen Umfangs-Absatz (384) aufweist, der sich nach außen hin von dem oberen Abschnitt an einer Stelle des oberen Abschnitts, die von dem offenen Ende des oberen Abschnitts räumlich beabstandet ist, erstreckt und wobei ein jedes Röhrchen weiterhin eine verformbare Kappe (364) aufweist, um eine gasdichte Abdichtung darauf auszubilden, umfassend:

   (a) ein einstückiges Einsatz-Element (342), das umfaßt:

      (i) einen flachen, horizontalen Platten-Abschnitt (372), der Ausnehmungen (374) in einer Anordnung einschließt, die mit einem Industriestandard-Mikrotiter-Format kompatibel ist, vorzugsweise 96 Ausnehmungen in einer 8 x 12 rechtwinkligen Anordnung, wobei die Ausnehmungen ein klein wenig größer sind als die Außendurchmesser der oberen Abschnitte der Röhrchen, jedoch kleiner als der Außendurchmesser jenes Absatzes,
      (ii) einen ersten vertikalen Einsatz-Seitenwandabschnitt (346) vollkommen um den Einsatz herum und sich nach oben hin erstreckend und zwar bis zu einer Höhe, die die Höhe eines Röhrchens, das in einer der Ausnehmungen aufsitzt, übersteigt,
      (iii) einen zweiten vertikalen Einsatz-Seitenwandabschnitt (346) um den Einsatz herum und sich nach unten hin erstreckend und zwar annähernd bis zu dem unteren Teil des oberen Abschnitts eines Röhrchens, das in einer der Ausnehmungen aufsitzt,

   (b) einen einstückigen Halter (386), der wiederversiegelbar im Innern des Einsatzes (342) in Eingriff genommen werden kann und zwar über jedwede Proberöhrchen, die in jenem Einsatz aufsitzen, wobei der einstückige Halter umfaßt:

      (i) einen flachen horizontalen Platten-Abschnitt (402), der Ausnehmungen (410) in einer Anordnung einschließt, die mit einem Industriestandard-Mikrotiter-Format kompatibel ist, vorzugsweise 96 Ausnehmungen in einer 8 x 12 rechtwinkligen Anordnung, wobei die Ausnehmungen ein klein wenig größer sind als die Außendurchmesser der oberen Abschnitte der Röhrchen, jedoch kleiner als der Außendurchmesser jenes Absatzes,
      (ii) einen vertikalen Halter-Seitenwandabschnitt um den Halter-Platten-Abschnitt herum und sich nach unten hin von jener Platte erstreckend,

   wobei, und zwar dann, wenn der Halter (386) im Innern des Einsatzes (342) in Eingriff ist, der Halter-Platten-Abschnitt ein klein wenig oberhalb des Absatzes eines Röhrchens, das in jenem Einsatz aufsitzt, liegt und der erste Einsatz-Wandflächen-Abschnitt in etwa so hoch ist, wie der Halter-Seitenwand-Abschnitt, wodurch die in jenem Einsatz aufsitzenden Röhrchen sowohl vertikal als auch lateral lose gehalten werden und die Kappen über den ersten vertikalen Einsatz-Seitenwand-Abschnitt hervorstehen, jedoch nach unten hin auf die Höhe des

Abschnitts verformbar sind.

2. Halter nach Anspruch 1, in welchem die Ausnehmungen in dem Einsatzabschnitt versenkt sind und in welchem die Unterseite der Absätze jener Röhrchen korrespondierend abgeschrägt sind.

3. Halter nach Anspruch 2, in welchem die Ausnehmungen in dem Einsatz-Platten-Abschnitt und in dem Halter-Platten-Abschnitt im Durchmesser größer sind als jene Röhrchen und zwar um 0,7 mm.

4. Halter nach einem Ansprüche 1 bis 3, in welchem das Einsatz-Element ferner eine Vielzahl von Stütz-Rippen umfaßt, die sich entlang der Unterseite des Einsatz-Platten-Elements zwischen Reihen von Ausnehmungen erstrecken, wobei die Rippen (406, 408) sich nach unten hin in dem gleichen Ausmaße hin erstrecken wie der zweite vertikale Einsatz-Seitenwand-Abschnitt.

5. Halter nach einem der vorhergehenden Ansprüche, in welchem das Einsatzelement weiterhin einen Randabschitt (366) umfaßt, der sich wenigstens teilweise um den Einsatz-Platten-Abschnitt herum erstreckt, und vertikal von diesem Abschnitt herabhängt, wobei der Randabschitt angepaßt ist, um in ein Schutzband (78) Rille in einem Thermozykler-Probenblock (12) zu passen.

6. Halter nach einem der vorhergehenden Ansprüche, in welchem der Einsatz-Platten-Abschnitt wenigstens zwei hierin bereitgestellte Öffnungen (416, 418) aufweist, und jener Halter-Platten-Abschnitt eine identische Anzahl von vertikalen Laschen (414A, 414B) aufweist, die sich von der Halter-Platte nach unten hin erstrecken, und zwar derart, daß die Laschen durch jene Öffnungen hindurchgehen und lösbar den Einsatz in Eingriff nehmen, wenn der Halter mit dem Einsatz zusammengesetzt wird.

7. Halter nach Anspruch 6, in welchem jene Laschen derart angeordnet sind, daß sie einen Teil des Randabschnitts bilden, der sich nach unten hin wenigstens teilweise um den Einsatz-Platten-Abschnitt herum erstreckt und in welchem die Laschen angepaßt sind, um in eine Schutzband-Rille in einem Thermozykler-Probenblock zu passen.

8. Halter nach Anspruch 7, in welchem die Öffnungen und die Laschen derart angeordnet sind, daß der Halter und der Einsatz zu lediglich einer Orientierung im Verhältnis zueinander in der Lage sind, wenn die Öffnungen und die Laschen in Eingriff sind.

9. Halter nach einem der Ansprüche 6 bis 8, in welchem die Laschen ablenkbar in einer Querbewegungsrichtung sind, um in Anstoß mit den Öffnungen zu gelangen.

10. Halter nach einem der vorhergehenden Ansprüche, der weiterhin Mikrotiter-Proben-Röhrchen in dem Halter umfaßt, und zwar vorzugsweise bis zu 96 Röhrchen.

11. Halter nach einem der vorhergehenden Ansprüche, in welchem eine jede Kappe einen nach unten herabhängenden, zylindrischen Flansch aufweist, um eine gasdichte Abdichtung mit einem der Röhrchen auszubilden sowie einen Umkreis-Absatz, der sich nach außen von dem Flansch erstreckt und der verhindert, daß der Flansch auf dem Röhrchen unterhalb eines vorbestimmten Punktes aufsitzt.

12. Halter nach Anspruch 11, in welchem der Außenumfang des nach unten herabhängenden Flansches satt anliegt um eine gasdichte Abdichtung mit dem Innenumfang des Röhrchens zu bilden.

13. Halter nach einem der vorhergehenden Ansprüche, in welchem 12-Gruppen jener Kappen miteinander verbunden sind, um einen einzelnen Streifen von Kappen auszubilden, die auf geeignete Weise beabstandet sind, um gasdichte Abdichtungen mit bis zu 12 jener Röhrchen auszubilden.

14. Halter nach einem der vorhergehenden Ansprüche, in welchem die Kappen deformierbar sind durch Hitze und einer nach abwärts gerichteten Kraft.

15. Halter nach einem der vorhergehenden Ansprüche, in welchem die Kappen elastisch deformierbar sind.

16. Halter nach einem der vorhergehenden Ansprüche der weiterhin einen Plastiksockel (420) umfaßt, der in einer Reihe angeordnete Bohrungen aufweist, vorzugsweise 96 Bohrungen in einer rechtwinkligen Reihe, wobei die Bohrungen derart dimensioniert sind, um die unteren Abschnitte der Probenröhrchen satt aufzunehmen, vorzugsweise bis zu 96 Röhrchen, wobei der Sockel mit dem Einsatz, dem Halter und den Röhrchen zusammensetzbar ist, um eine Mikrotiter-Platte auszubilden, die den Fußabdruck einer Industrie-Standard-Mikrotiter-Platte aufweist.

Revendications

1. Dispositif de maintien en plastique (342, 386) constitué de deux pièces pour maintenir librement des tubes d'échantillon pour microtitrage (376) d'une configuration présélectionnée, de préférence jusqu'à 96 tubes d'échantillon, ayant chacun une section supérieure (288) de forme cylindrique ouverte

à son extrémité supérieure et une section inférieure rétrécie, fermée, s'étendant vers le bas depuis cette dernière, chaque tube étant de section transversale circulaire et ayant un épaulement circonférentiel (384) s'étendant vers l'extérieur depuis ladite section supérieure au niveau d'une position sur ladite section supérieure espacée de ladite extrémité ouverte de celle-ci, chaque tube ayant en outre un capuchon déformable (364) pour former un joint étanche au gaz sur celui-ci, comprenant :

a) un organe formant plateau (342) constitué d'une pièce comprenant :

i) une section de plaque horizontale, plane (372) comprenant des trous (374) en un réseau compatible avec les formats de plaque pour microtitrage selon les normes de l'industrie, de préférence 96 trous en un réseau rectangulaire 8 fois 12, lesdits trous étant légèrement plus grands que le diamètre extérieur des sections supérieures desdits tubes mais plus petits que le diamètre extérieur dudit épaulement,
ii) une première section de paroi latérale verticale du plateau (346) entourant complètement ladite plaque et s'étendant vers le haut jusqu'à une hauteur supérieure à la hauteur d'un tube reposant dans l'un desdits trous,
iii) une seconde section de paroi latérale verticale du plateau (346) entourant ladite plaque et s'étendant vers le bas approximativement jusqu'au fond de la section supérieure d'un tube reposant dans l'un desdits trous,

b) un dispositif de retenue (386) constitué d'une seule pièce engageable de manière libérable à l'intérieur dudit plateau (342) sur n'importe quel tube d'échantillon reposant dans ledit plateau, comprenant :

i) une section de plaque horizontale plane (402) comprenant des trous (410) en un réseau compatible avec les formats de plaque pour microtitrage selon les normes de l'industrie, de préférence 96 trous en un réseau rectangulaire 8 fois 12, lesdits trous étant légèrement plus grands que le diamètre extérieur des sections supérieures desdits tubes mais plus petits que le diamètre extérieur dudit épaulement,
ii) une section de paroi latérale verticale du dispositif de retenue entourant ladite section de plaque du dispositif de retenue et s'étendant vers le haut depuis ladite plaque,

dans lequel, lorsque ledit dispositif de retenue (386) est engagé à l'intérieur dudit plateau (342), la section de plaque du dispositif de retenue s'étend légèrement au-dessus de l'épaulement d'un tube reposant dans ledit plateau et la première section de paroi latérale du plateau est environ aussi haute que ladite section de paroi latérale du dispositif de retenue, de sorte que les tubes reposant dans ledit plateau sont retenus librement à la fois verticalement et latéralement et lesdits capuchons sont en saillie au-dessus de la première section de paroi latérale verticale du plateau mais sont déformables vers le bas jusqu'à la hauteur de ladite section.

2. Dispositif de maintien selon la revendication 1, dans lequel les trous dans ladite section du plateau présentent une contre-dépouille et dans lequel les faces inférieures des épaulements desdits tubes sont inclinées de manière correspondante.

3. Dispositif de maintien selon la revendication 2, dans lequel les trous dans la section de plaque du plateau et dans la section de plaque du dispositif de retenue sont plus grands en diamètre que lesdits tubes d'environ 0,7 mm.

4. Dispositif de maintien selon l'une quelconque des revendications 1 à 3, dans lequel ledit organe formant plateau comprend en outre plusieurs nervures de support s'étendant le long de la face inférieure de l'organe formant plaque du plateau entre des rangées de trous, lesdites nervures (406, 408) s'étendant vers le bas sur la même hauteur que lesdites secondes sections de paroi latérale verticale du plateau.

5. Dispositif de maintien selon l'une quelconque des revendications précédentes, dans lequel ledit organe formant plateau comprend en outre une section de jupe (366) s'étendant au moins partiellement autour de ladite section de plaque du plateau et étant suspendue verticalement depuis cette section, ladite section de jupe étant adaptée pour s'ajuster dans une rainure de bande de garde (18) dans un bloc d'échantillons de thermocycleur (12).

6. Dispositif de maintien selon l'une quelconque des revendications précédentes, dans lequel ladite section de plaque du plateau comprend au moins deux ouvertures (416, 418) prévues dans celle-ci et ladite section de plaque du dispositif de retenue a un nombre identique de pattes verticales (414A, 414B), s'étendant vers le bas depuis ladite plaque du dispositif de retenue, de telle manière que lesdites pattes sont en saillie au travers desdites ouvertures et coopèrent de manière libérable avec le plateau lorsque ledit dispositif de retenue est assemblé avec ledit plateau.

7. Dispositif de maintien selon la revendication 6, dans lequel lesdites pattes sont disposées de manière à former une partie d'une section de jupe s'étendant vers le bas au moins partiellement autour de ladite section de plaques du plateau et dans lequel lesdites pattes sont adaptées pour s'ajuster dans une rainure de bande de garde dans un bloc d'échantillon de thermocycleur.

8. Dispositif de maintien selon la revendication 7, dans lequel lesdites ouvertures et lesdites pattes sont positionnées de telle manière que ledit dispositif de retenue et ledit plateau peuvent avoir seulement une orientation relative l'un par rapport à l'autre lorsque lesdites ouvertures et lesdites pattes coopèrent.

9. Dispositif de maintien selon l'une quelconque des revendications 6 à 8, dans lequel lesdites pattes peuvent être défléchies dans une direction latérale de manière à venir en alignement avec lesdites ouvertures.

10. Dispositif de maintien selon l'une quelconque des revendications précédentes, comprenant en outre des tubes d'échantillon pour microtitrage dans ledit dispositif de maintien, de préférence jusqu'à 96 tubes.

11. Dispositif de maintien selon l'une quelconque des revendications précédentes, dans lequel chacun desdits capuchons comprend une bride cylindrique suspendue vers le bas pour former un joint étanche au gaz avec l'un desdits tubes et un épaulement circonférentiel s'étendant vers l'extérieur depuis ladite bride qui empêche que ladite bride soit reçue sur le tube en dessous d'un point prédéterminé.

12. Dispositif de maintien selon la revendication 11, dans lequel la circonférence extérieure de la bride suspendue vers le bas s'ajuste avec un frottement doux pour former un joint étanche au gaz avec la circonférence intérieure dudit tube.

13. Dispositif de maintien selon l'une quelconque des revendications précédentes, dans lequel des groupes de douze desdits capuchons sont joints ensemble pour former un cordon unique de capuchons qui sont espacés de manière adaptée pour former des joints étanches au gaz avec jusqu'à douze desdits tubes.

14. Dispositif de maintien selon l'une quelconque des revendications précédentes, dans lequel lesdits capuchons sont déformables avec la chaleur et une force verticale orientée vers le bas.

15. Dispositif de maintien selon l'une quelconque des revendications précédentes, dans lequel lesdits capuchons sont déformables de manière élastique.

16. Dispositif de maintien selon l'une quelconque des revendications précédentes, comprenant en outre une base en plastique (420) ayant des puits agencés en un réseau, de préférence 96 puits en un réseau rectangulaire, lesdits puits étant dimensionnés de manière à recevoir avec un frottement doux les sections inférieures desdits tubes d'échantillon, de préférence jusqu'à 96 tubes, ladite base pouvant être assemblée avec ledit plateau, ledit dispositif de retenue et lesdits tubes pour former une plaque pour microtitrage ayant l'empreinte d'une plaque pour microtitrage selon les normes de l'industrie.

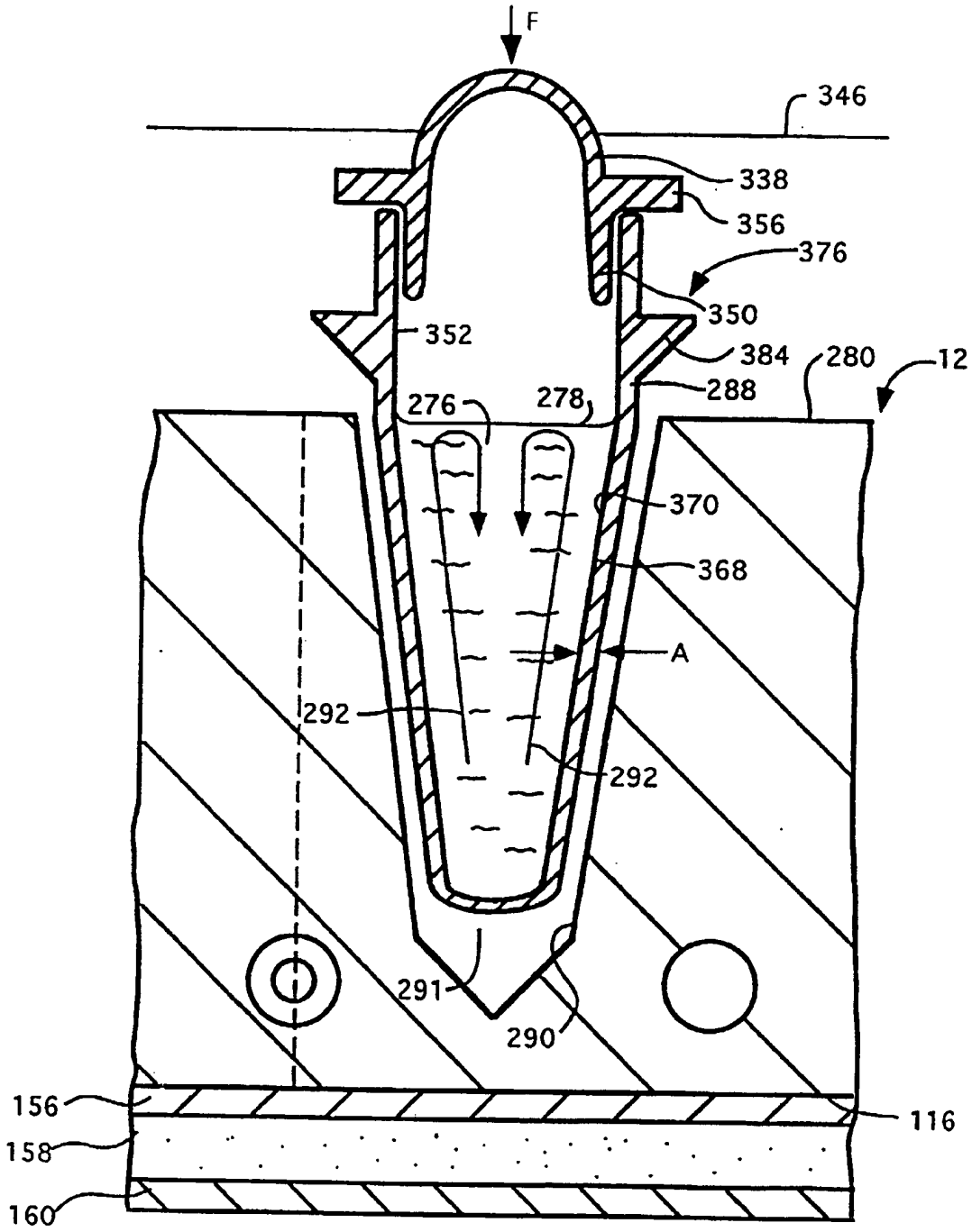
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FIG. 1

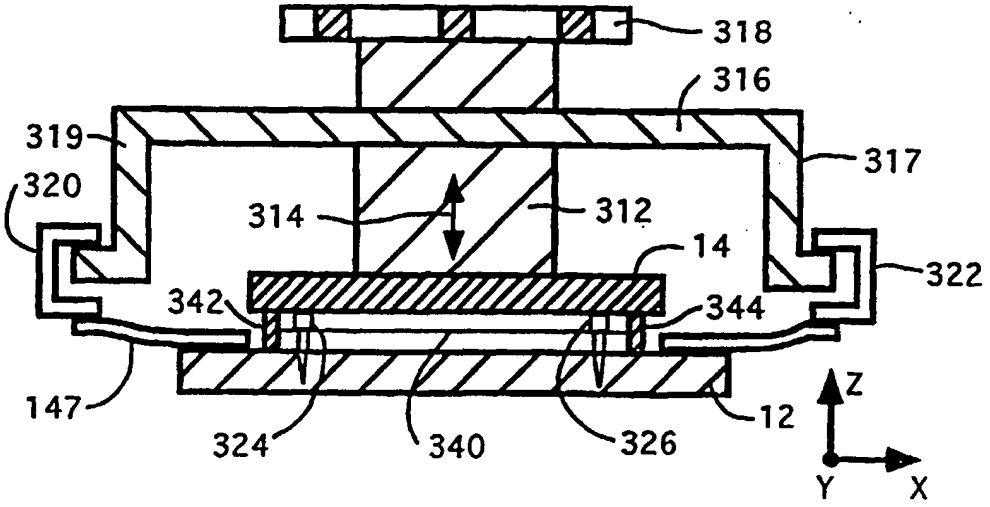
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FIG. 2

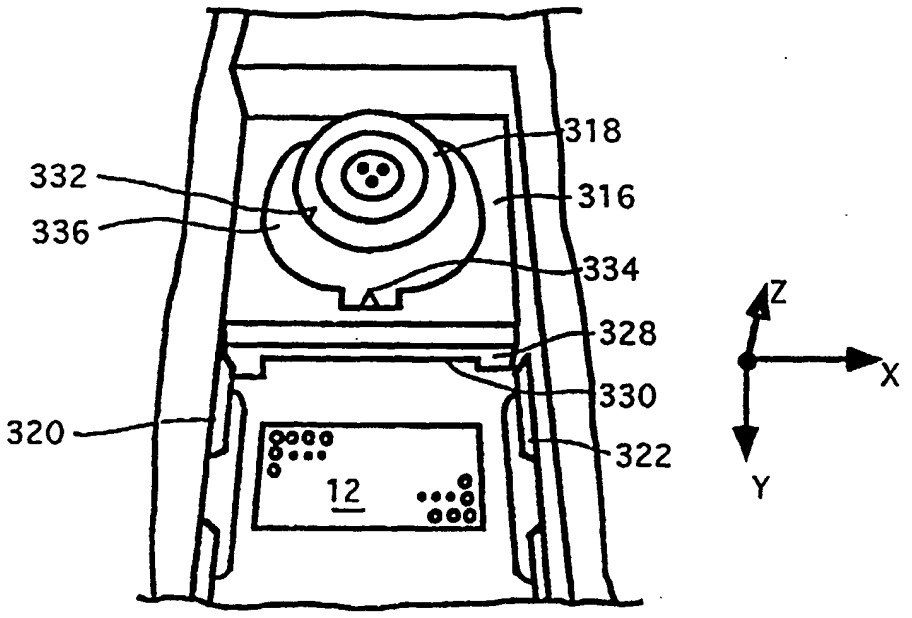
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FIG. 3

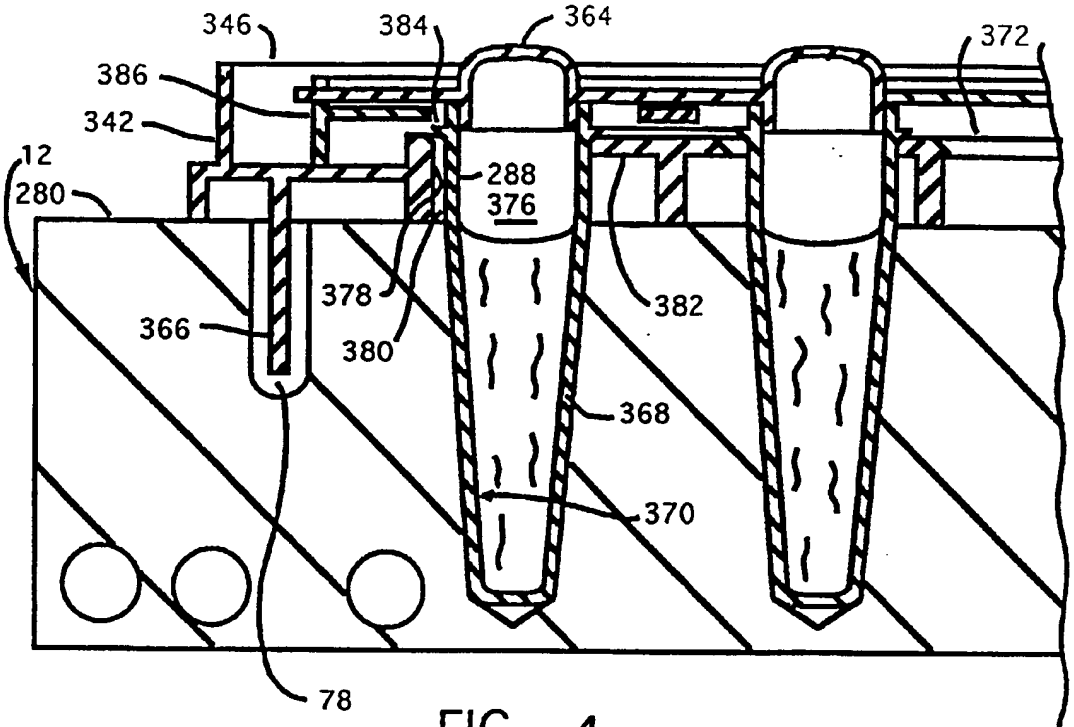
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FIG 4

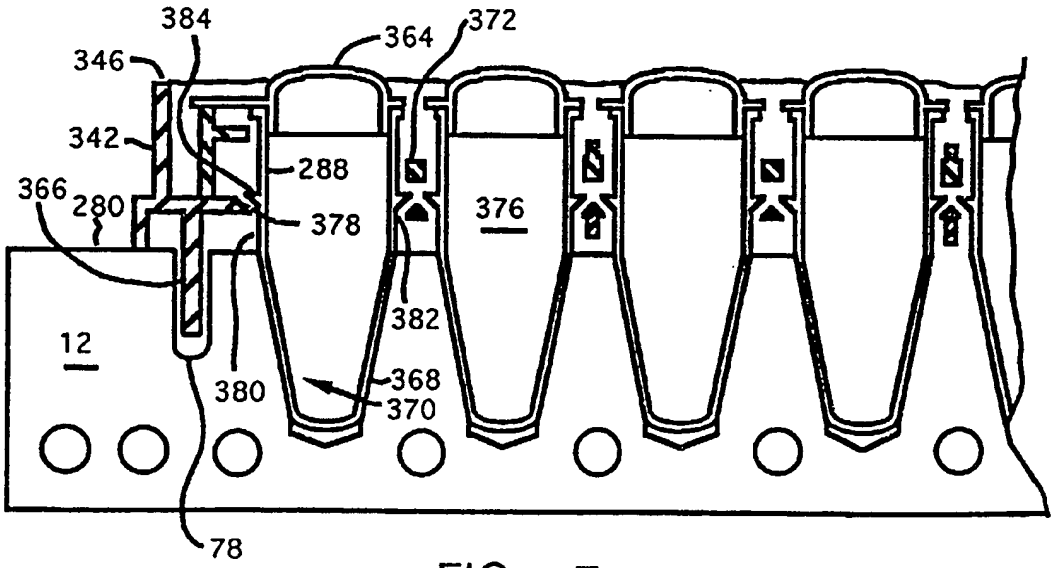
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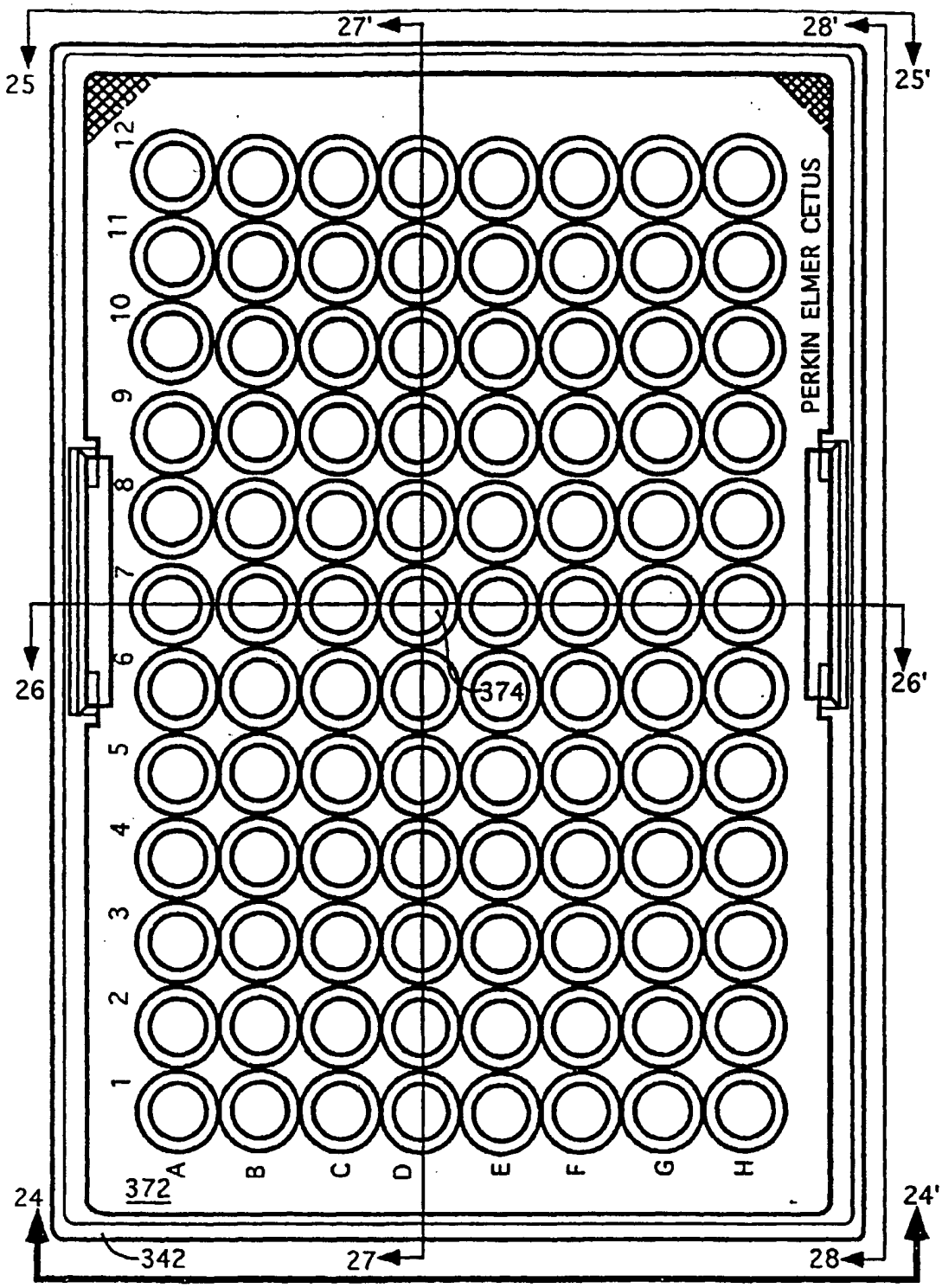
FIG. 5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25'
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6
5
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3
2
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PERKIN ELMER CETUS
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24'
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FIG. 6

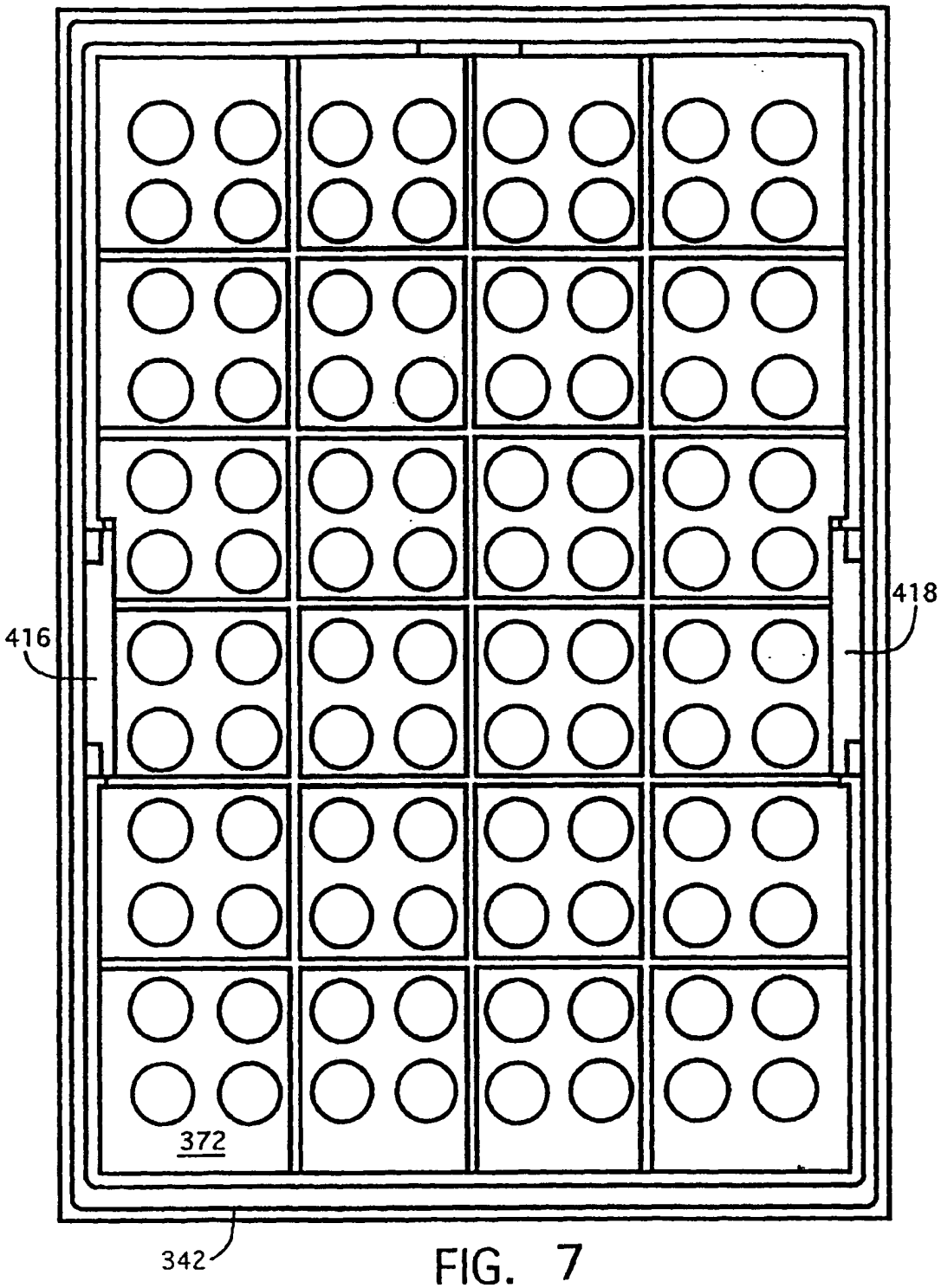

FIG. 7

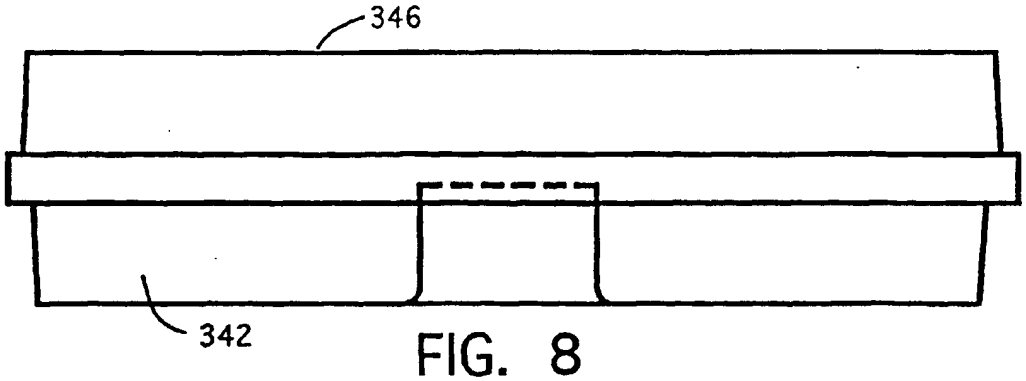

FIG. 8

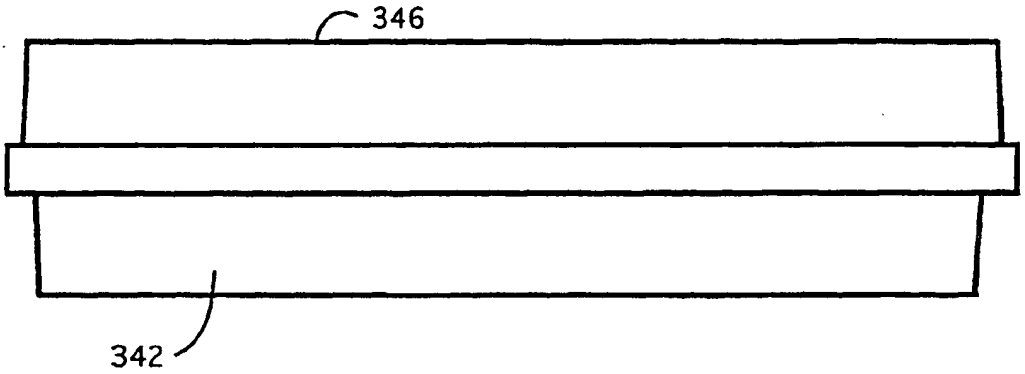

FIG. 9

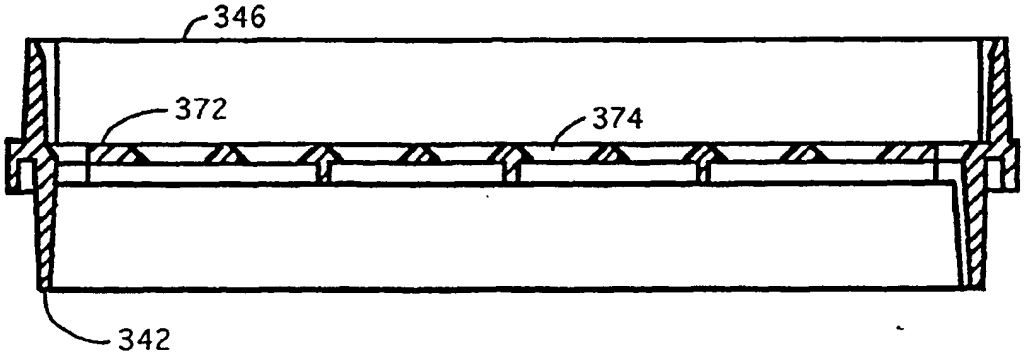

FIG. 10

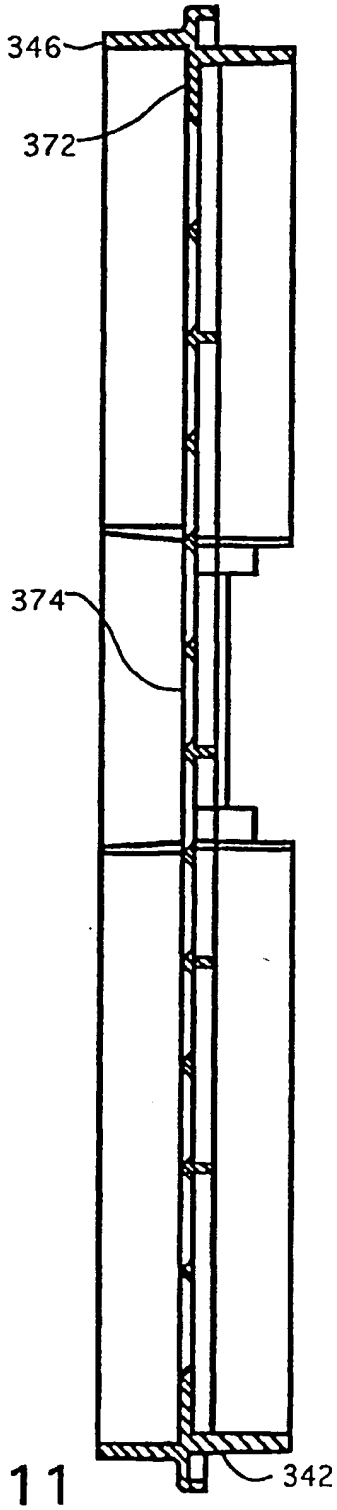

FIG. 11

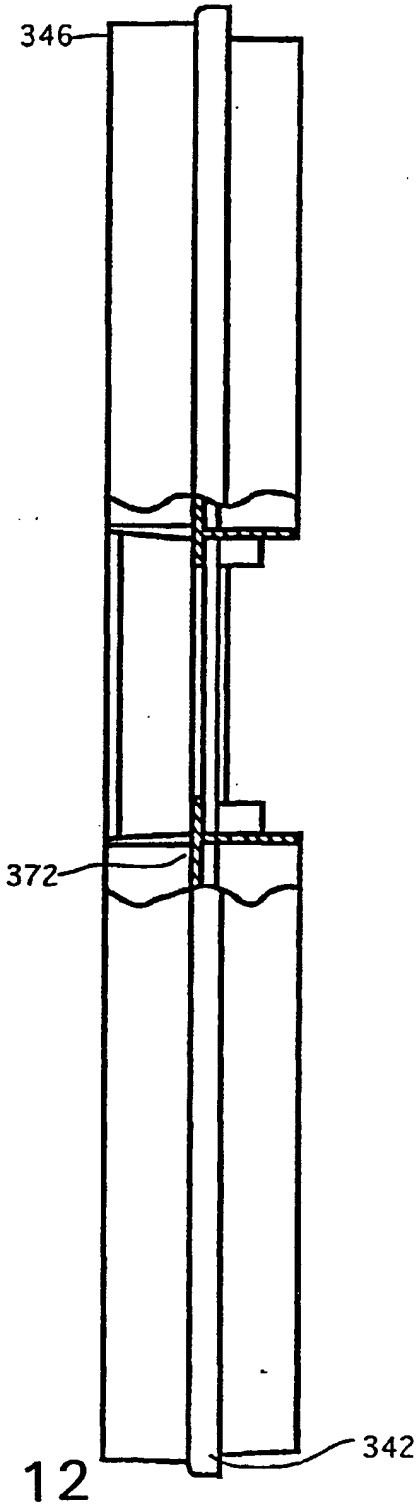

FIG. 12

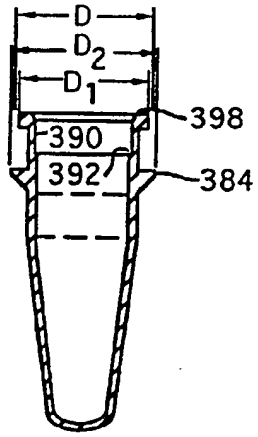

FIG 13

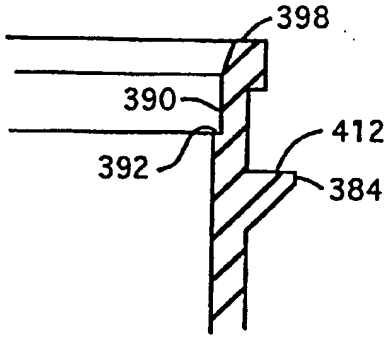

FIG 14

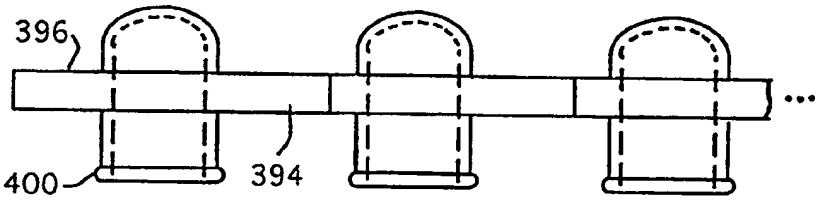

FIG 15

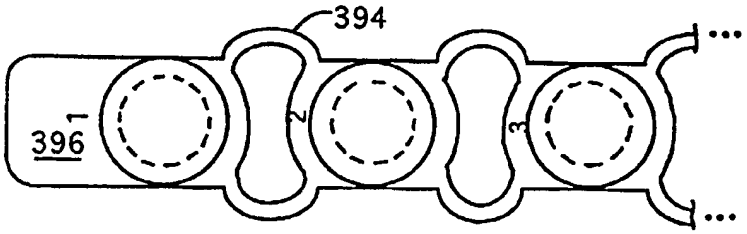

FIG 16

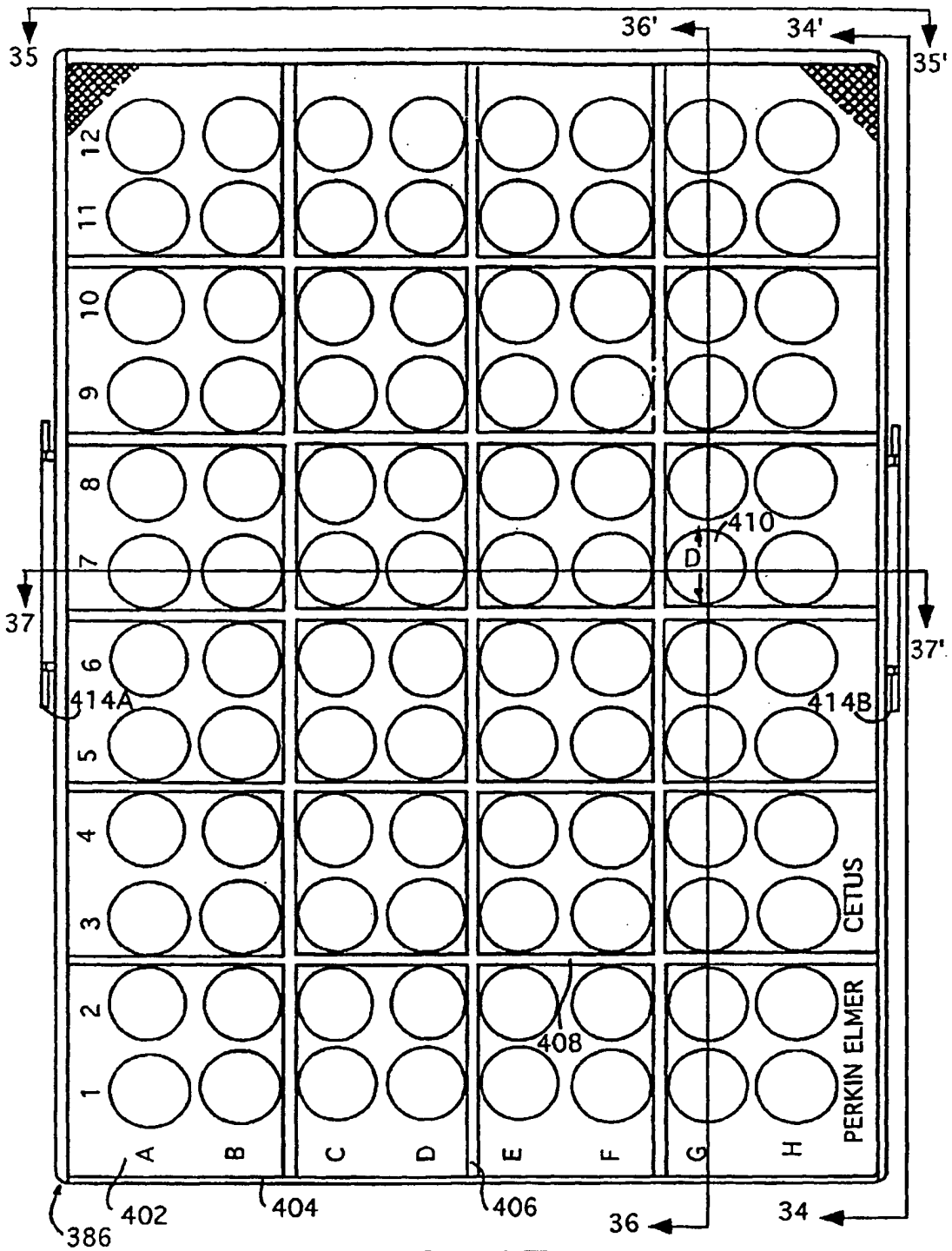
35
36'
34'
35'
12
11
10
9
8
7
6
5
4
3
2
1
410
D
37
37'
414A
414B
CETUS
PERKIN ELMER
408
A
B
C
D
E
F
G
H
386
402
404
406
36
34

FIG. 17

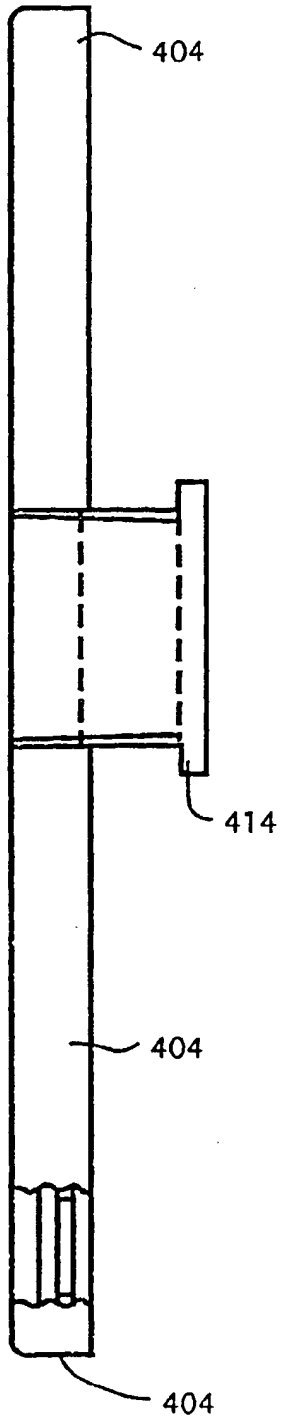

FIG. 18

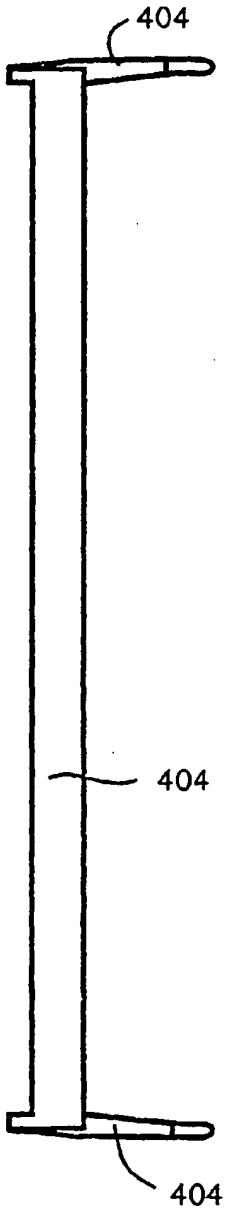

FIG. 19

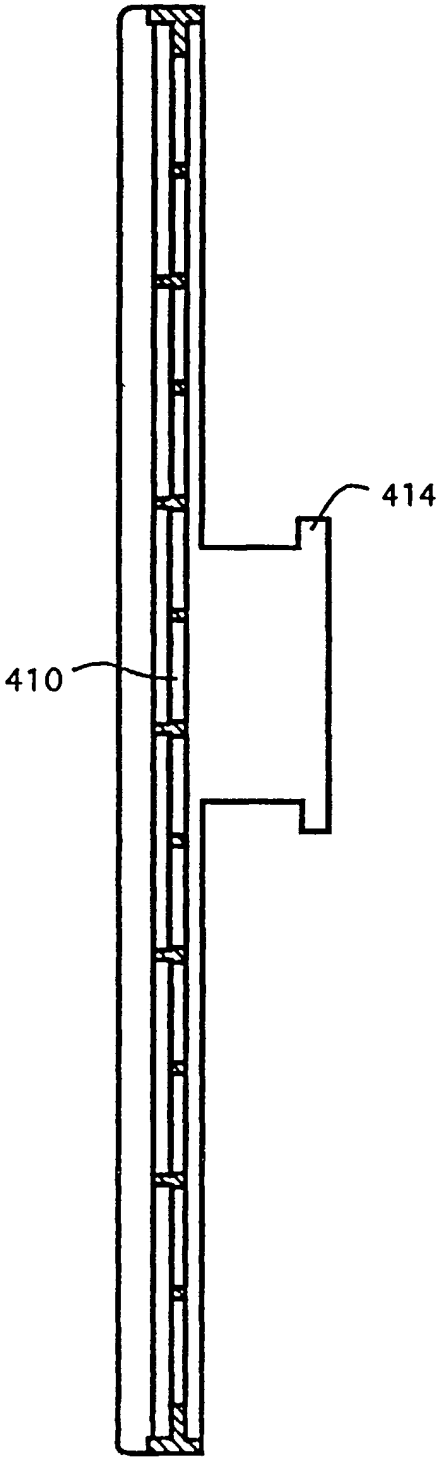

FIG. 20

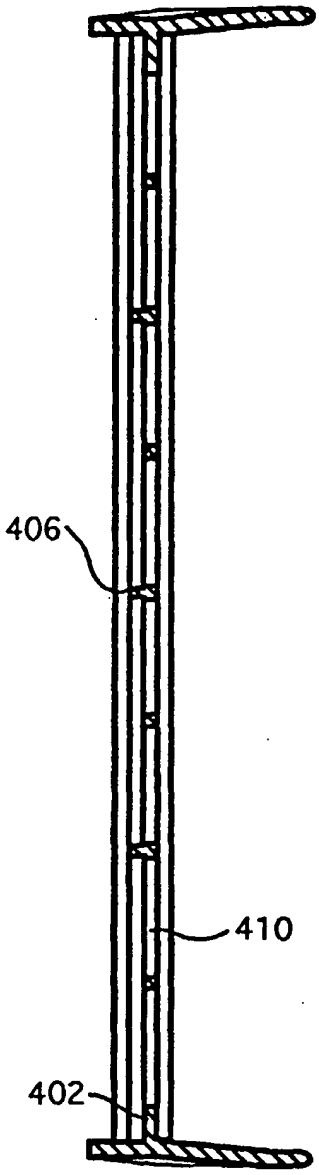

FIG. 21

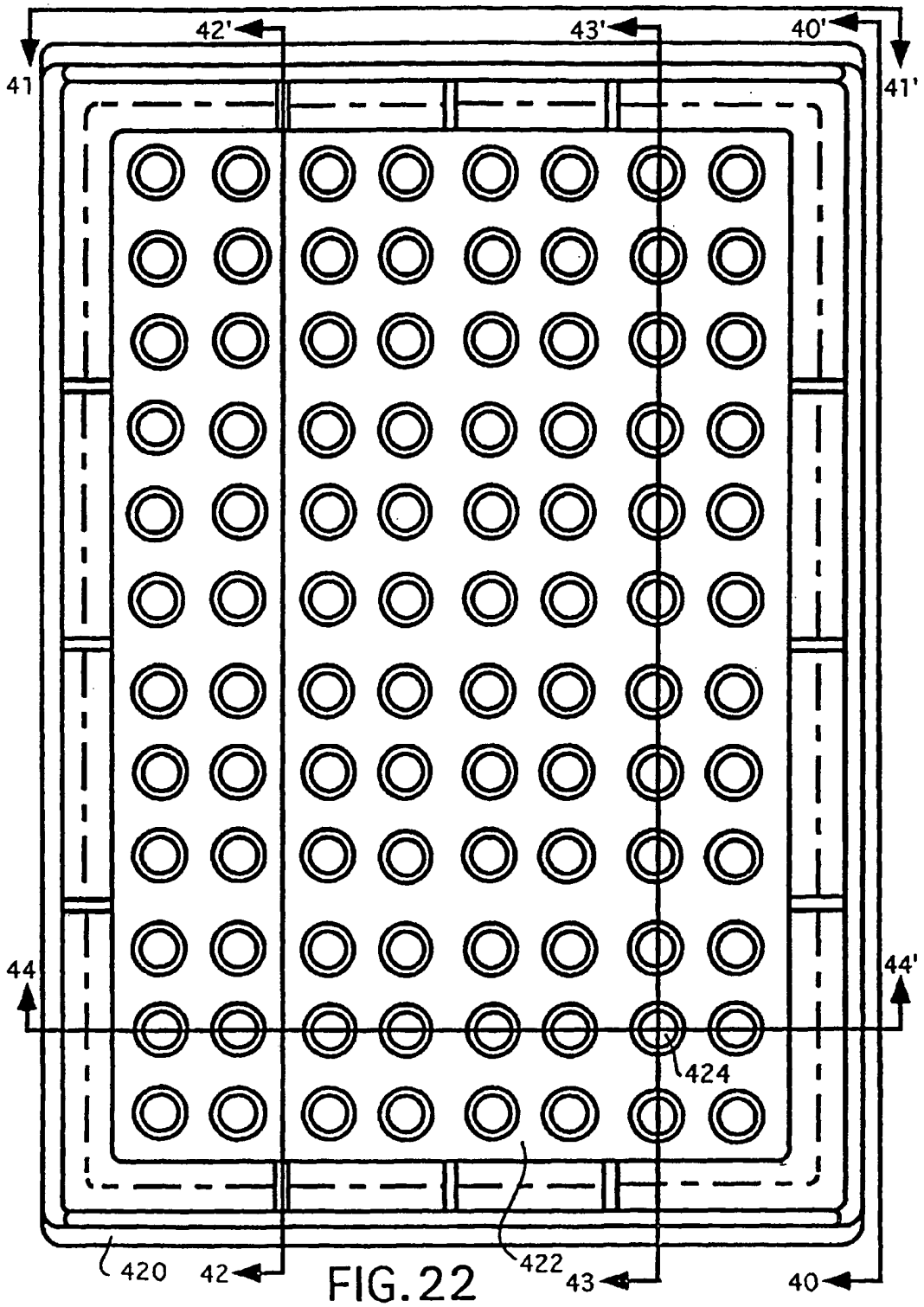

FIG. 22

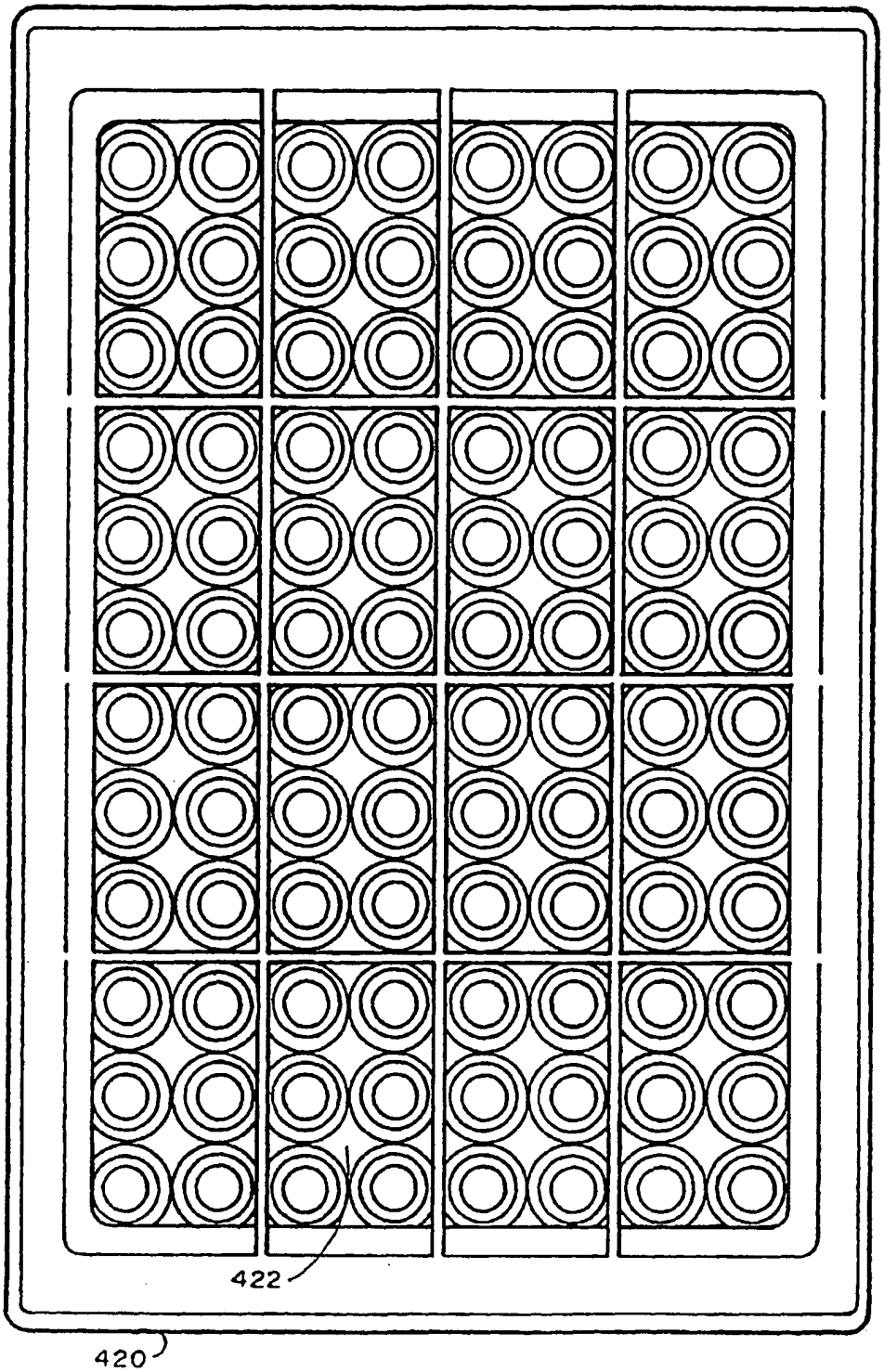

FIG. 23

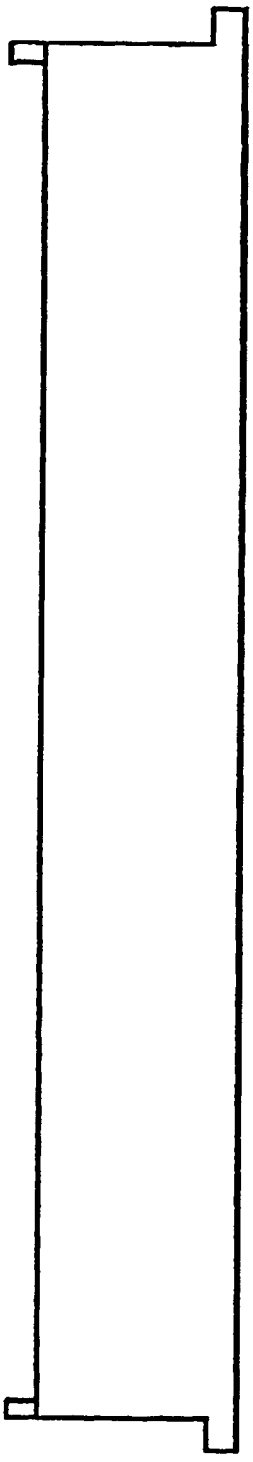

FIG. 24

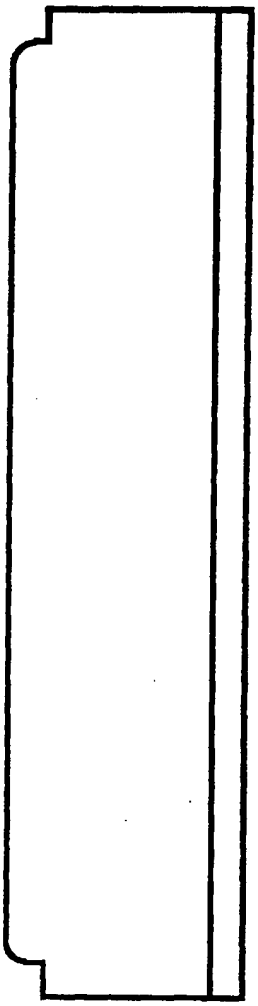

FIG. 25

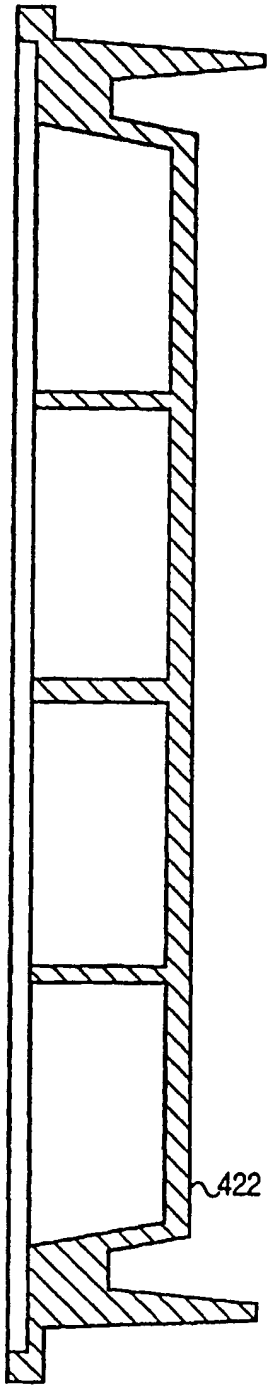

FIG. 26

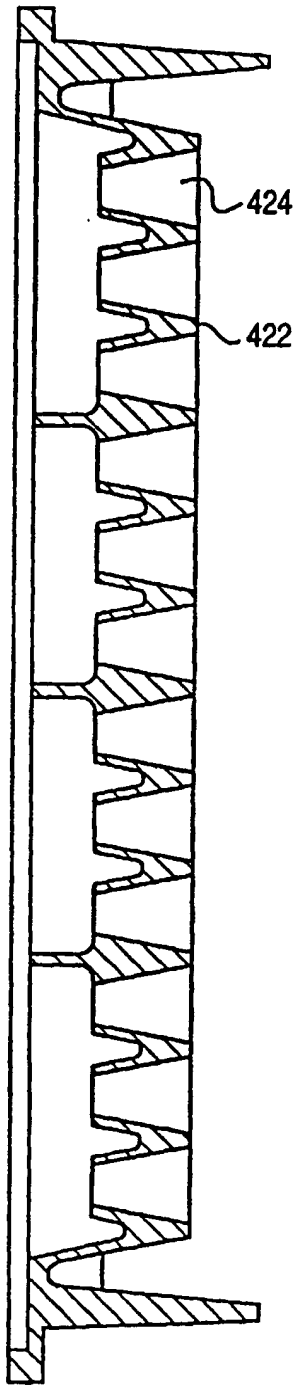

FIG. 27

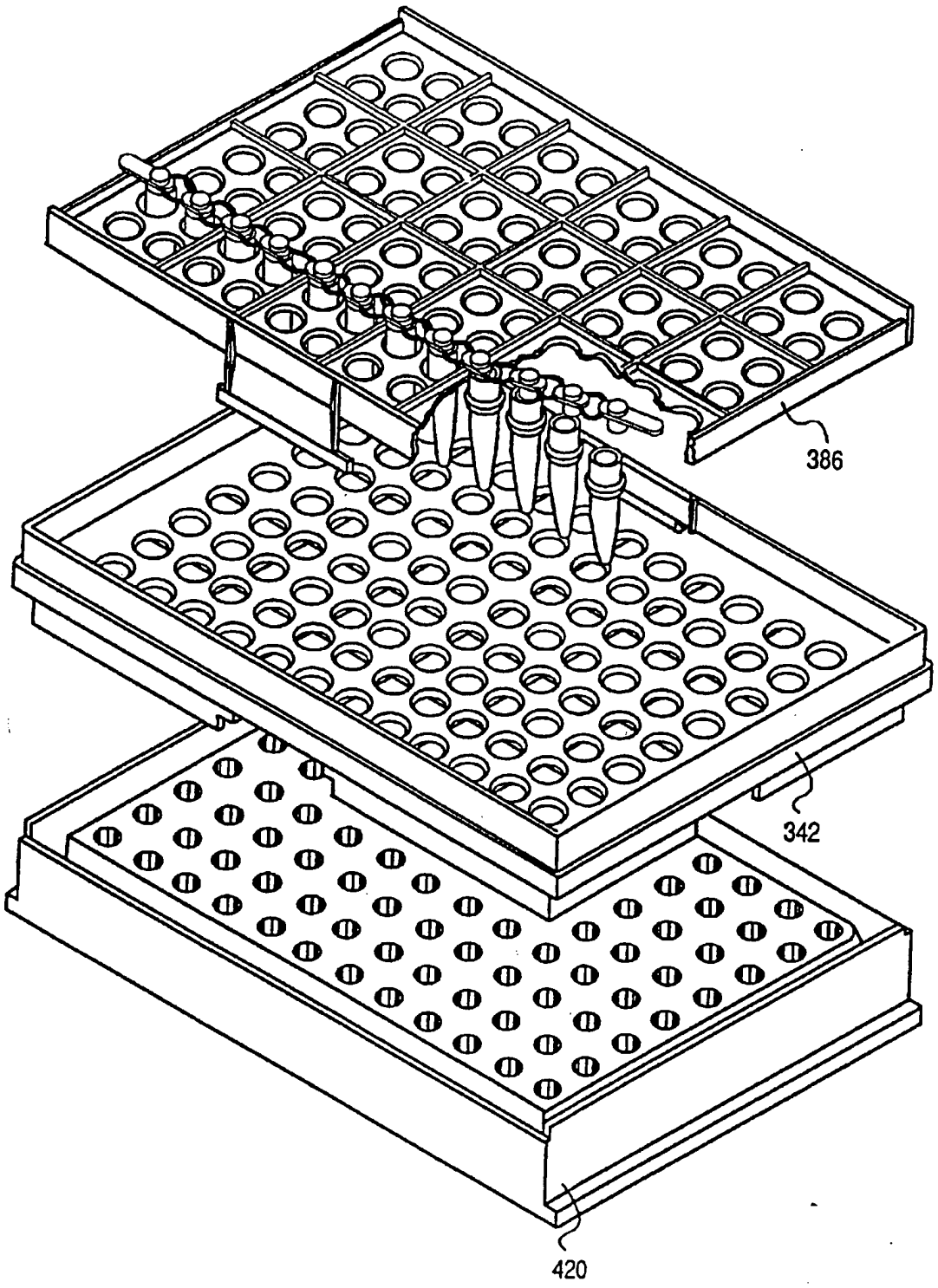

FIG. 28

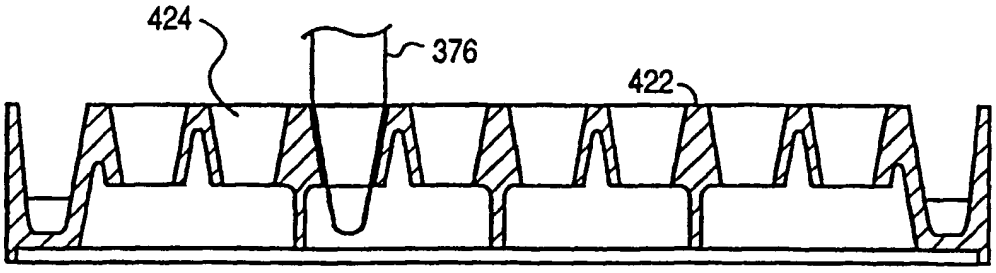

FIG. 29

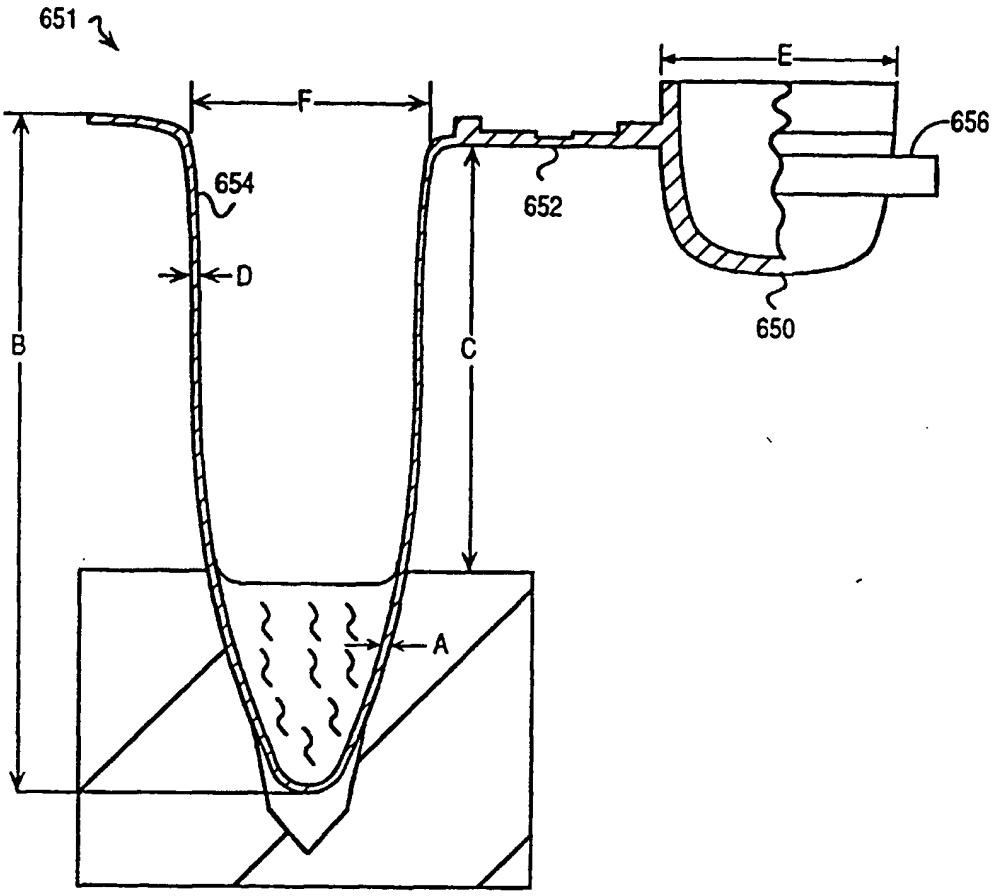

FIG. 30

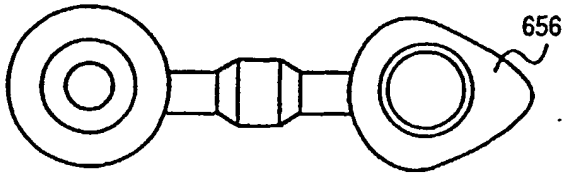

FIG. 31